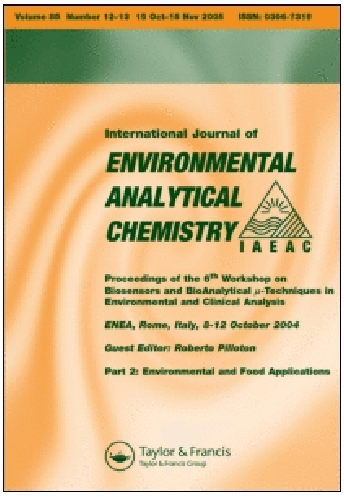

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INTERFERENCE OF HUMIC SUBSTANCES IN THE SPECTROPHOTOMETRIC DETERMINATION OF BROMATE BY PHENOTHIAZINES IN NATURAL WATERS

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The spectrophotometric method of bromate (BrO_3^-) determination by phenothiazines was applied to natural water samples and the interferences due to the presence of inorganic and humic substances were investigated. Common ions present in natural waters did not interfere and only the less abundant NO_2^- and Fe^{2+} exhibited strong interferences. Interferences of the two latter ions, if they existed, could be controlled and the method proved to be accurate and with a low detection limit. However, it was found that the presence of soluble humic substances resulted in positive interference, rendering the method unsuitable for bromate determination in natural waters and restricted its use in pure bromate solutions. This interference can be attributed to the electron acceptor groups invariably existing in the humic molecules. Since humic substances can remain in the water even after its ozonation, they will also contribute to a positive interference in bromate determination in potable waters.

Keywords: Bromate determination; phenothiazines; natural waters

INTRODUCTION

Bromate ion (BrO_3^-) is considered a hazardous substance and USEPA has established a concentration value of 10 μg/L as maximum contaminant level in waters. Normally, BrO_3^- is determined in potable waters that have undergone ozonation prior to their use, because it is a by-product of this process. In certain cases, however, it has importance to measure bromate and evaluate the analytical

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interferences in natural waters (streams, rivers) from the stand-point of assessing the degree of their potential pollution with bromate. Any information obtained about interfering substances is also of value, since most of them are not completely destroyed or do not disappear by the following sanitation treatment (e.g. ozonation) and can still interfere or cause problems in the subsequent analytical determination of bromate.

The importance of bromate to human health has resulted in the appearance of several publications during the last decade[1–7], dealing with the analytical methods of bromate determination in potable waters and the possible interferences. Since the established maximum contaminant level of bromate has been set at very low value (10 μg/L), all the proposed methods for bromate quantification focus in achieving high sensitivity and low detection limit. The commonly used method for bromate determination is ion chromatography (IC) which has been standardized and considered as a reference method. IC, however, is not free from weakness and difficulties, the main one being chloride interference[1–3]. Weinberg[1]overcame this problem by using a silver cation resin and a chelation column to remove leached silver, in order to protect the separation column. The detection limit of the method was 0.5 μg/L after the application of a pre-concentration technique. A similar procedure was followed by Joyce and Dhillon[2], who achieved a detection limit of 1 μg/L. IC separation with no pre-treatment, followed by a post-column reaction to produce tribromide from bromate with subsequent detection by UV absorbance at 267 nm, was proved a very sensitive method[4] with an experimental detection limit of 0.2 μg/L.

Due to certain difficulties involved in the IC method and its time consuming and complex nature, a simpler and faster spectrophotometric BrO_3^- determination was sought and proposed. The method used chlorpromazine[5] and other phenothiazines[6] as color-producing reagents and seemed an interesting and attractive alternative method. In acidic environment phenothiazines are oxidized by BrO_3^- to form stable, colored cations. The proposed method[5,6] had a low detection limit and no interference by Cl^- and other anions and cations commonly present in natural waters. Using either chlorpromazine (CPH) or triflupromazine (TFP) resulted in almost the same sensitivity and in strong interference by nitrite ion[6]. The interference of ClO_2^- was very pronounced with CPH and hardly evident with TPH. The use of 10H-phenothiazine (PH) resulted in lower sensitivity than when CPH and TFP were used, but the interference of NO_2^- and ClO_2^- was as high as with CPH. The theoretical details of the method have been thoroughly studied with pure bromate solutions[6]. The method, however, has not found practical application for bromate determination in real water samples. Only one case has been reported in which the method has been used in natural water analysis as post-column bromate determination following IC separation[7].

The objectives of this work were to apply the spectrophotometric method of BrO_3^- determination in natural water samples using phenothiazines and evaluate the interference of various substances present. The results were confirmed by using IC as a reference method.

EXPERIMENTAL

Spectrophotometric Determination

Sampling

Five natural water samples were collected from Northern Greece during May 1999 and will be referred as samples No 1–5 henceforth. Pretreatment of the samples included filtration through a 0.45 μm pore size membrane filter.

Instrumentation

A Lambda 2 UV/VIS version 3.7 Perkin Elmer spectrophotometer was used.

Reagents

Stock solutions of CPH, TFP and $KBrO_3$ were prepared by dissolving the appropriate amount of reagent in distilled water. Working standards were prepared by proper dilution of the stock solution. The sodium salt of humic acid (Aldrich, H1675–2, Lot Number 16308) was used for simulating the presence of soluble natural organic substances.

Procedure

A 50 mL sample was mixed with:

- 15 mL of 1000 mg/L CPH solution and 15 mL 6N HCl and after 5 min the absorbance was measured at 527 nm against distilled water in 1 cm pathlength measurement cells.
- 3 mL of 10^{-3} M TFP solution and 5 mL 6 N HCl and after 10 min the absorbance was measured at 504 nm against distilled water in 1 cm pathlength measurement cells.

Ion Chromatographic Determination

An IC Wescan model 213, with a conductivity detector (cell volume 0.5 μL), an HPLC Marathon II pump for the eluent and a SSI model 300 LC pump for the sample were used. Two 6-port rotary injection valves Rheodyne 7125 with loops of 100 μL and 10 mL were used for the sample injections. Conductivity suppression was achieved by using Alltech cation exchange columns, in H^+ form, fitted on a separate suppressor Alltech model 335. A Spectra Physics SP 4270 for chromatogram integration was used. An Alltech anion HC separation column and a Hamilton PRPX100 (with exchange capacity 0.19±0.02 meq/g) as a concentrator column were used. In addition, Guard Ag cartridges Alltech IC-Ag^+ for Cl^- removal were used. The eluent used was 2.8 mM $NaHCO_3$/2.2 mM Na_2CO_3 with flow rate equal to 1.2 mL/min. The injected sample volume was 5 mL followed by a pre-concentration step.

RESULTS AND DISCUSSION

Reference method

Certain comments concerning the IC reference method need to be made. The equation of the calibration curve, in the range of 1 – 10 μg/L, was $Y_{mm}=2.63X_{conc}-0.13$ (r=0.9997) and the detection limit was about 1 μg/L. This detection limit increased to 5 μg/L in the case of natural water samples due to chloride interference. Chloride gave a broadened peak after pre-concentration, although the samples were treated with IC-Ag cartridges in series for its removal. The method also showed low BrO_3^- recoveries in natural waters for concentrations below 10 μg/L, while the relative standard deviation was estimated to be 20% at 10 μg/L BrO_3^- and higher than 20% at lower BrO_3^- concentrations. These findings were in agreement with the remarks of Legube[8].

Spectrophotometric determination

The absorbance spectrum of a 100 μg/L BrO_3^- solution confirmed that the maximum absorbance of CPH appeared at 527 nm and that of TFP at 504 nm. Due to photosensitivity of blanks with phenothiazines, distilled water was used as a blank solution. The calibration curve was linear between 6 μg/L and 900 μg/L ($Y_{abs}=0.72\times10^{-4}X_{conc}+8.25\times10^{-4}$, r=0.9996) for TFP and between 4 and 600 μg/L BrO_3^- ($Y_{abs}=1.9\times10^{-4}X_{conc}+1.9\times10^{-4}$, r=0.9999) for CPH. The detection limit of the method, defined as 3σ of 10 replicates, was equal to 3.5 μg/L BrO_3^- for TFP and 2 μg/L BrO_3^- for CPH.

The experimental results showed that the presence of common ions in water such as Na^+, K^+, Ca^{2+}, Mg^{2+}, NO_3^-, SO_4^{2-} and Cl^-, in concentrations up to

500 mg/L, did not interfere in the bromate determination with both phenothiazines, which is in agreement with the results of Gordon et al.[5], and Farrel et al.[6] Nitrite exhibited strong interference, which is also in agreement with the observations of Farrel et al.[6]. Trace elements that are commonly present in natural waters, such as F^-, I^-, Br^-, Mn^{2+}, Zn^{2+}, Cr^{3+}, Sr^{2+}, Al^{3+} and B in concentrations up to 500 µg/L did not interfere. The only interfering cation was Fe^{2+}. This interference was negative (Table I, column 2), because Fe^{2+} eliminates BrO_3^- ions through their reduction. It is self-evident, however, that when BrO_3^- is measured in ozonated waters, this interference is of no importance, since Fe^{2+} does not exist.

TABLE I Effect of Fe^{2+} added to: a) 50 µg/L BrO_3^- solution and b) 2 mg/L Humic Acid (HA) solution

Fe^{2+} added, µg/L	*BrO_3^- measured[a] in 50 µg/L solution*	*µg/L Pseudo-BrO_3^- concentration[a] in 2mg/L HA solution*
0	50	20
50	50	21
100	43	23
200	23	
300	19	28
400	18	
500	5	26

a. measured by CPH and TFP.

Whenever the phenothiazines method was applied for BrO_3^- determination in natural waters, high concentrations were measured (Table II, column 2), in spite of the fact that these samples had not been treated with ozone or any other disinfection method. Bromate was measured in the same water samples by the IC reference method, in order to confirm the accuracy of the spectrophotometric method. The results, however, showed bromate concentrations below the detection limit (Table II, column 5). Consequently, it was obvious that BrO_3^- concentration measured by the phenothiazine-method represented pseudo-concentrations and the presence of some agent which causes interference was suspected. This positive interference was additive to known amounts of standard BrO_3^- in all natural water samples tested, as it is shown in Table II, columns 3 and 4.

Since none of the main and trace elements, present in natural waters and mentioned previously, justified this BrO_3^- pseudo-concentration values, the only parameter left to be examined was the soluble humic substances, which are

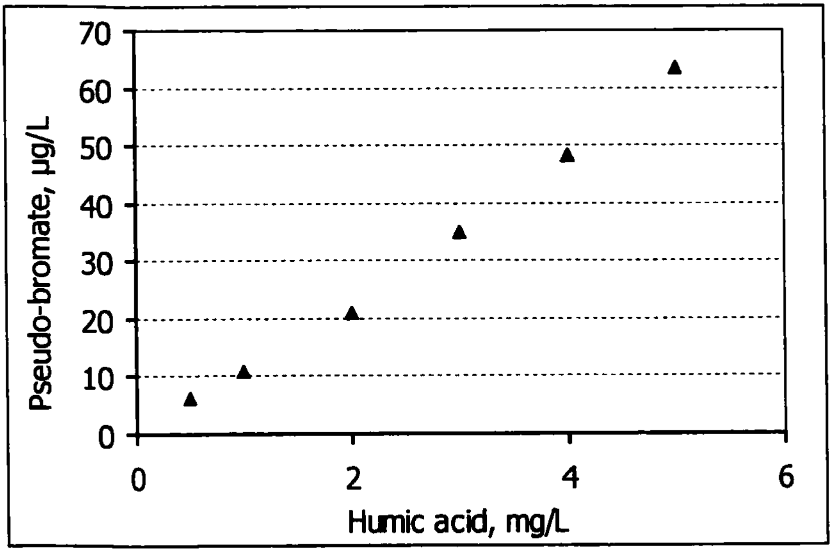

FIGURE 1 Pseudo-bromate concentration manifested by various, bromate-free, humic acid solutions, when the phenothiazine method was applied

always present in natural waters. Their presence was confirmed by obtaining the absorbance spectrum of the samples in the ultraviolet range (190–300 nm). The spectrum showed the absence of distinct peaks and a gradual increase in absorbance as the wavelength decreases, which is characteristic of the soluble humic substances. After this finding, humic acid (HA) solutions, for simulating the presence of natural organic substances, were prepared and used for testing their behavior. The results showed that HA solutions interfered in bromate determination giving pseudo-concentration values proportional to their concentration (Fig. 1). This interference was additive, as verified when a known amount of bromate was added in a HA solution (Table II, last row). The same additive interference was also observed in natural waters (Table II, rows 2–6). Moreover, there seemed to be an interaction of Fe^{2+} with HA, because, when different concentrations of Fe^{2+} were added to 2 mg/L HA solution, an increased bromate pseudo-concentration was observed, as the amount of added Fe^{2+} increased (Table I, column 3). Similar behavior of Fe^{2+} was observed in natural waters (Fig. 2). This is contrary to the expected, because Fe^{2+} should have a negative interference on bromate concentration, as mentioned previously and found with pure bromate solutions (Table I, column 2).

TABLE II BrO_3^- concentrations (μg/L) measured in natural water samples by CPH and IC methods

	Results by CPH method[a]			
Sample	*Measured*	*Added*	*Found*	*IC*
Distilled water	0	100	100	BDL[b]
1	13	100	111	BDL
2	62	100	164	BDL
3	30	100	126	BDL
4	50	100	148	BDL
5	48	100	147	BDL
2 mg/L HA solution	20	100	118	BDL

a. Similar results were obtained by TFP method.

b. Below detection limit.

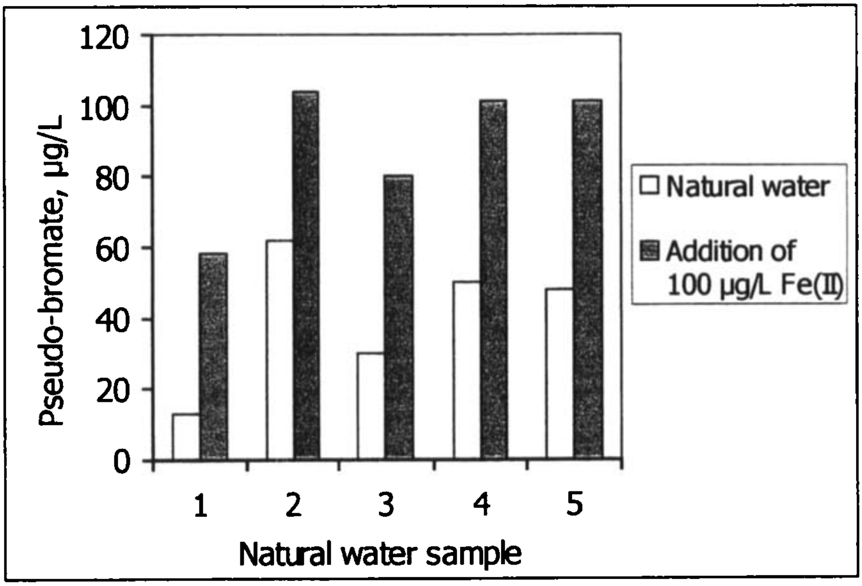

FIGURE 2 Effect of Fe^{2+} on pseudo-bromate concentrations measured by CPH in natural water samples

The interference of humic substances with the bromate determination can be attributed to the following mechanisms. It is known that phenothiazines (an oxidizable substrate-electron donor) are converted to the highly persistent, colored radical cations in the presence of an electron acceptor (oxidant) through a reversible electron transfer process[6,9], shown in the simplified scheme of Figure 3. The electron acceptor can be either any substance containing electron acceptor groups or an element and polyatomic ion with unoccupied electronic orbitals. The most prominent example of the latter case is the nitrite ion.

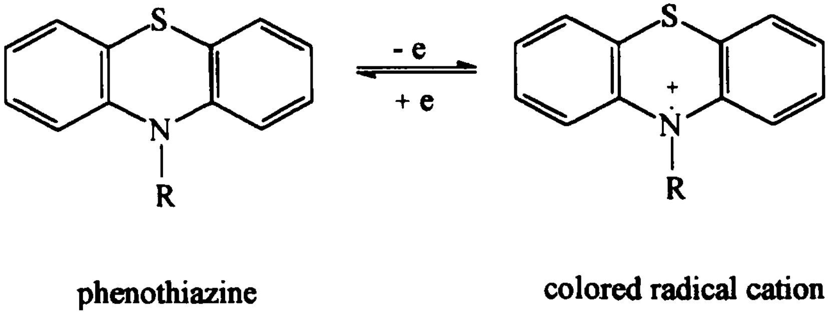

FIGURE 3 Single electron transfer process for converting phenothiazine to its radical cation

Humic substances contain electron acceptor groups, such as C=O, COOH and quinone-like free radicals with an unpaired electron which has a strong tendency to be paired[10]. Therefore, electron-deficient quinone-like structures in the humic substances can induce a single electron transfer from the electron-rich nitrogen atom of the phenothiazine molecule, giving rise to the formation of colored radical cation[11], which could subsequently interfere with the bromate determination.

The other possible mechanism by which humic substances can serve as electron acceptor agents is through the involvement of certain metallic cations, such as iron, which is a common constituent of the humic molecule in coordination with organic ligands. These elements are not necessarily fully coordinated and can still have unoccupied electronic orbitals. Therefore, they attract and share one electron with the nitrogen atom of the phenothiazines forming inner-sphere complexes in acidic media[12]. This can lead to the formation of the colored phenothiazine cation radical.

In conclusion, it was confirmed that the significant parameter, which strongly interfered in bromate determination by phenothiazines in natural waters, was the naturally occurring humic substances. These substances can persist in the water after its ozonation and will contribute to a positive interference in bromate determination in drinking waters.

Acknowledgements

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