J Fluoresc (2008) 18:1169–1179
DOI 10.1007/s10895-008-0369-3

ORIGINAL PAPER

Online Monitoring of Bromate in Ozonized Water Without a Previous Separation Process

M. J. Almendral-Parra · A. Alonso-Mateos · M. S. Fuentes-Prieto

Received: 18 February 2008 / Accepted: 14 March 2008 / Published online: 24 July 2008

Abstract The use of ozonation for the purification of drinking water can lead to the formation of bromate. The US Environmental Protection Agency and the European Directive for human drinking water has lowered the regulatory level for bromate down to 10 μg l^{-1}, such that methods must be developed for monitoring the formation of bromate, particularly in on-site situations. In the present work we report a fluorometric method for the determination of bromate based on the reaction with carbostyril-124, a compound that shows florescence mainly at pH values above 4 and, when bromated, generates a non-fluorescent product. The reaction can thus be used as an indirect method for determination of the ion. The proposed method, which uses the flow injection (FI) technique, allows online application and kinetic control of the variables affecting the process, together with shorter reaction times, and it provides maximum sensitivity and selectivity. Under optimum conditions, it is possible to determine the analyte within the 4–200 μg l^{-1} range, with a limit of detection of 0.9 μg l^{-1} and a relative standard deviation (n=12, $[BrO_3^-]$= 5 and 30 μg l^{-1}) of 3.2% and 2.6% respectively. The determination rate was ten samples per hour.

Keywords Bromate · Water · Fluorimetry · Flow injection analysis · Carbostyril-124 · Disinfection by-product

M. J. Almendral-Parra (✉) · A. Alonso-Mateos · M. S. Fuentes-Prieto
Departamento de Química Analítica, Nutrición y Bromatología, Faculty of Chemistry, University of Salamanca, Plaza de la Merced s/n, 37008 Salamanca, Spain
e-mail: almendral@usal.es

Introduction

Water destined for human consumption must be subjected to a series of treatments aimed at eliminating pathogenic agents and reducing other pollutants to acceptable levels that are not harmful to health. In this process, chemical treatment—and within this disinfection—is important. The disinfection process involves different physical, biological and chemical treatments. The latter can be performed with chlorinated products (chlorine, chloramines, chlorine dioxide), ozone, potassium permanganate, quaternary ammonium salts, etc. In its different forms, chlorine is the chemical agent most widely used. Although the attention of many researchers has focused on the by-products of chlorine disinfection, other chemical disinfecting agents also generate by-products when they react with organic substances and other precursors present in untreated water. Bromate, for instance, is essentially a by-product of the ozonation of water with high bromide contents [1, 2] and amounts between 60–90 μg l^{-1} have been found in ozonized water.

Chronic exposure to $KBrO_3$ causes kidney cell tumors in rats, hamsters and mice, and thyroid and testicular mesothelial tumors in rats [3–5]. Owing to its cross-species carcinogenicity, bromate is considered a probable human carcinogen by the International Agency for Research on Cancer (IARC) [6]. It also has a potential negative effect on health, which can damage the nervous system, kidney and thyroid gland [7]. European Directive 98/83 /CEE of 3 November 1998 on the quality of water intended for human consumption establishes that the maximum contaminant level (MCL) of bromate in drinking water should be 10 μg l^{-1} [8] as from 2009, accepting values of up to 25 μg l^{-1} for the period between 2004 and 2008. This gives countries the chance to achieve lower values, when possible, without this affecting disinfection quality. This guideline value is provisional because of limitations in available analytical and treatment methods, and there is an urgent need to set up techniques able to detect levels of 2.5 μg l^{-1} or even lower. The US Environmental Protection Agency (US EPA) has proposed to set the maximum contaminant level for bromate ion in drinking water at 10 μg l^{-1} [9]. In order to control this value in real samples, fast automated analysis systems are required.

The literature contains many references to methods for the analysis of bromate in matrices with concentrations ranging from μg l^{-1} to mg l^{-1}. According to the US EPA., during the nineties, the maximum pollutant level expected lay between 0.1 and 1 mg l^{-1}. The first methods were developed to meet this objective but since the limit value of bromate was reduced to 10 μg l^{-1} during the past decade the development of new methods has focused on improving sensitivity [10].

Ion chromatography (IC) with conductivity detection (IC–CD) is the technique most widely employed. The determination of bromate by means of this technique has a limit of detection of 20 μg l^{-1} (method 300.0, 1989 of the EPA [11]). Using a chromatographic system with preconcentration, it is possible to attain a limit of detection of 1.3 μg l^{-1}. This, however, has the drawback of being subject to the action of many interferents (such as chloride) and requires steps prior to sample introduction into the instrumental system, which prolongs analysis times. The method must be improved as regards the identification and pretreatment of samples. Recently, a new ion exchange column has been developed that seems to overcome the problems of interference and does not require a preconcentration step. The sensitivity of the technique has been increased in two ways: improving the technology of the columns (lowering the detection limit to 1.3 μg l^{-1} (method 300.1, 1997, [12]) and performing sample pretreatment to remove the main interferents (standard ISO 15061 method [13]), attaining a limit of detection of 0.5 μg l^{-1}. The method has been modified by post-column reaction with KBr-*o*-dianisidine reagents [14], and the removal of ClO_2 with Fe(II) (EPA Method 317.0) [15]. A bromate-specific method (EPA Method 324.0) [16] and post-column reaction methods (EPA Method 326.0) [17, 18] have also been proposed.

Inductively coupled plasma–mass spectrometry (ICP–MS) can be coupled with IC [19–21] or flow injection systems [22], affording a detection limit of 0.1–0.3 μg l^{-1} of bromate, which can be further reduced to 0.05 μg l^{-1}. Moreover, some analytical methods based on spectrophotometric detection after post-column reaction [14, 23, 24], gas chromatography [25], chemiluminescence detection [26] and electrospray IC-tandem mass spectrometry [27, 28] have also been reported for the determination of bromate [29].

Few fluorimetric methods have been reported for the determination of bromate, among them ion chromatography with a fluorescence detector using the post-column reaction of the bromation of carbostyril-124 (CARB) in acid medium. This method has two pitfalls: the need to use a very long reactor, thus extending the analysis time, and an important degree of interferences from the ClO_2^- ion. The quantification rate is 1.6 μg l^{-1} [10].

Although these techniques meet the requirements of current legislation, they are relatively complex, time consuming and require high-technology and expensive instrumentation; they are thus not suited for routine on-site monitoring. In order to monitor ozonation and to control bromate in drinking water on-site, simple, low-cost and robust methods are necessary.

Among the non-chromatographic methods, spectrofluorimetry is a sensitive technique that can be readily adapted for online determinations. Here we report a fluorimetric procedure for bromate determination based on the above carbostyril reaction, with no a previous separation process, using flow injection analysis (online application) and kinetic control of the variables affecting the reaction, achieving shorter reactions times with maximum sensitivity and selectivity.

Experimental

Apparatus and materials

Minipuls HP4 (Gilson, France) peristaltic pumps with silicone or vinyl pump tubes. PTFE simple-injection valve (Rheodyne, model 5020). Detection was performed with an RF-5000 spectrofluorimeter (Shimadzu, Japan) fitted with a DR-15 data processor and an FDU-13 data storage unit, to which a sensitization unit was coupled, (Shimadzu, 200-26841-01). A 25-μl flow cell (Hellma, Germany, 176.052) with an optical pathway of 0.150 cm and a rectangular quartz flow cell (Shimadzu, 204–05566) of 1 cm optical pathway and 12 μl volume were employed. PTFE tubing of 0.5 mm internal diameter with standard tube fittings and connectors (Upchurch Scientific, Inc.) was used. A Crison 501 potentiometer and a Digiterm 3000542 (Selecta) water bath thermostatted at 27 °C were also used.

Reagents and solutions

All chemicals used in this work were of analytical grade and were prepared with ultra-high quality deionized water. 5.02×10^{-4} M solution of carbostyril-124 (CARB, Aldrich) prepared by weighing the reagent and dissolution in distilled water, to which 20.0 ml of 12 M HCl (Panreac) and 10 g of solid KBr (Carlo Erba) had been added.

Solution 1.26 M of sodium acetate (Prolabo). Standard concentrated solutions of 1.0017 g l^{-1} of potassium bromate, $KBrO_3$, (Panreac) and of 0.4200 g l^{-1} of sodium chlorite, $NaClO_2$, (Acros organics). Working standard solutions were prepared after suitable dilutions of the stock solution with distilled water. Solutions of a large number of inorganic ions prepared from their water-soluble salts were also used.

Flow System

Figure 1a shows the flow scheme proposed for the determination of BrO_3^- by means of the CARB bromation reaction and fluorimetric measurement of the excess reagent after the reaction.

The device has three channels and two peristaltic pumps. Channel C_1 carries the CARB reagent dissolved in HCl medium, at a strongly acid pH and in the presence of excess KBr. Channel C_2 carries a constant flow of bidistilled H_2O, which acts as carrier, into which a volume of standard solution or sample containing BrO_3^- is injected. Both solutions merge at a confluence point (T connection) where the bromation reaction of the CARB reagent begins, this consisting of two steps: the reaction between BrO_3^- and Br^- to generate bromine and the bromation of the CARB reagent.

From the first point of confluence to the second one, the reaction time is controlled, together with the flow rates F_R and F_P, with a 0.5 mm inner diameter Teflon reactor of variable length. In order to control the reaction temperature, the reactor is placed in a thermostatted water bath. Channel C_3 carries a continuous flow of a solution of NaAc, with a flow rate of F_A, at a suitable concentration, so that when channels C_1 and C_2 meet the resulting pH will be appropriate for the measurement of the fluorescence generated by the excess CARB from the bromation reaction (λ_{exc}=339 nm, λ_{em}=430 nm). The excitation and emission slit widths are maintained at 10 nm at all times. Throughout the study a flow cell of 25 µl was employed; this was introduced into the fluorimeter in a sensitization unit for greater optical efficiency (with concave mirrors).

Once the chemical variables and the flow rate had been optimized, in order to study the rest of the geometric and hydrodynamic variables a practical modification of the flow scheme depicted in Fig. 1 was made (Fig. 1b). A peristaltic pump controlled the flow rate of the carrier solution, F_P, which was kept at a value of 0.38 ml min^{-1}, while with the second peristaltic pump F_R and F_A were controlled at the same time; these were kept at the same value of 0.10 ml min^{-1}. This new system allowed the flow rate at which the sample was injected to be independent of the flow rate of the reagents, allowing sample dilution to be controlled. Using this flow system, the analytical calibrations were performed, together with the study of interferences and validation of the method.

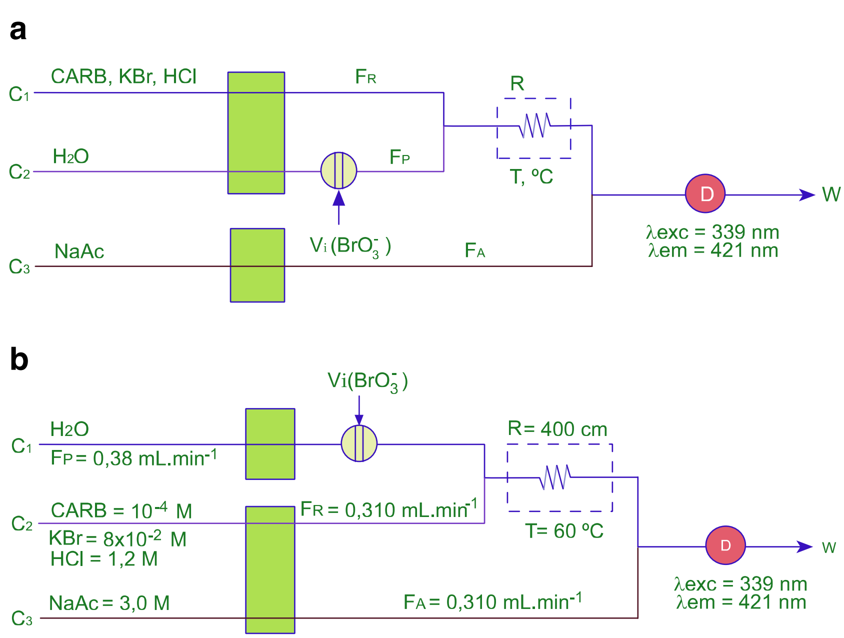

Fig. 1 **a** Flow scheme proponed for the determination of BrO_3^- in continuous mode by means of the CARB bromation reaction. Fluorimetric determination of the excess reagent. *CARB* carbostyril-124, F_R, F_P and F_A flow rates of the solutions carried through C_1, C_2 and C_3, V_i injection volume, *R* reactor, *D* fluorimeter. **b** Optimum flow scheme for the determination of BrO_3^- by means of the carbostyril-124 bromation reaction

Results and discussion

Fluorescence spectra

The 7-amino-4-methyl-2-hydroxyquinoleine: $C_{10}H_{20}N_2O$ is commercialized under different names. One of them, used in the proposed method, is carbostyril-124, a compound that is insoluble in HCl and that mainly shows fluorescence at pH values higher than 4 and that, upon bromation, generates a non-fluorescent product. This reaction is therefore used as an indirect method for the determination of BrO_3^- after separation by ion chromatography. The decrease in fluorescence is proportional to the concentration of BrO_3^-.

Figure 2 shows the excitation spectra of CARB at pH 4.8 at the optimum λ_{em} (430 nm) and the emission spectra, at the same pH value, obtained with the optimum λ_{ex} (339 nm). The same solution of CARB, but at more acid pH values, afforded the same spectra but with lower fluorescence intensities, fluorescence disappearing at pH values below 2. The protonated forms did not emit fluorescence. Above a pH of 4.3, the fluorescence remained constant, owing to the predominance of the basic form of the organic molecule.

When KBr and excess $KBrO_3$ (excess bromation) were added to an acid solution containing CARB, adjusting pH to 4.8, the final solution did not show fluorescence because the bromation product formed was not fluorescent (bromation on carbons with double bonds).

When designing the flow scheme the pH conditions for the bromation reaction (a strongly acid medium) and the pH zone for the measurement of the fluorescence of the excess reagent, with a pH above 4.3, were taken into account.

The solutions of CARB in HCl medium were stable for at least 8 days after their preparation. This was checked by measuring fluorescence during that period of time and conserving them under refrigeration.

Figure 3 shows the analytical signal obtained upon injecting in duplicate a standard solution of BrO_3^- under suitable experimental conditions. The baseline corresponds to the emission of fluorescence by the CARB reagent at the pH of the second point of confluence (>4.3). When a volume of a standard solution of BrO_3^- was injected, it reacted with the Br^- contained in the reagent solution at acid pH, generating Br_2, which reacted with CARB to form a non-fluorescent compound. The analytical signal generated was negative, since it measured the decrease in fluorescence intensity due to the decrease in the CARB concentration. The height of the fiagram, ΔI_F, was directly proportional to the concentration of the oxidant, BrO_3^-.

Preliminary studies

Kinetic characteristics of the reaction

Influence of reactor length In light of the difficulty involved in performing a kinetic study of the reaction in discontinuous mode, owing to the problem of the difference in the optimum pH for the chemical reaction and for fluorescence emission, kinetic data concerning the chemical reaction were obtained by operating in continuous mode, since this is performed at a preset time with the possibility of controlling both pH values simultaneously.

In the flow scheme depicted in Fig. 1a the following conditions were imposed: Channel C_1: $[CARB]=10^{-4}$ M, $[KBr]=6.7\times10^{-2}$ M, $[HCl]=0.8$ M, $F_R=0.24$ ml min^{-1}; Channel C_2: Water as carrier, $V_i=243$ µl of a standard solution of BrO_3^- at a concentration of 200 µg l^{-1}, $F_P=$ 0.24 ml min^{-1}; Channel C_3: $[NaAc]=2.5$ M, $F_A=0.24$ ml min^{-1}. Although these were not optimum conditions, it was possible to guarantee a sufficiently acid pH for the BrO_3^-–Br^- reaction to occur, and the high NaAc concentration ensured that the pH of the second confluence point was

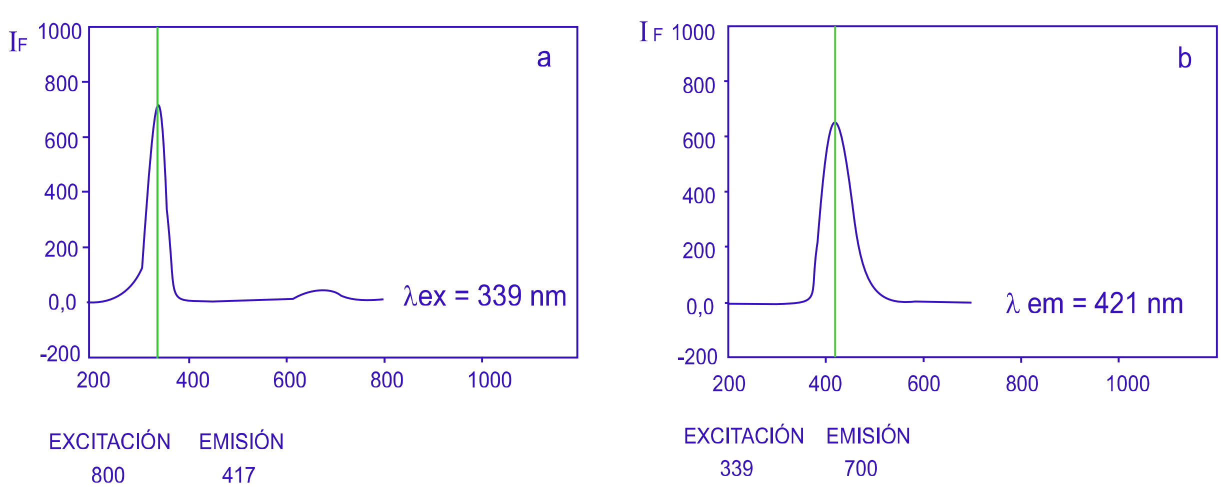

Fig. 2 Spectra **a** excitation, **b** emission, of carbostyril-124 at pH 4.8. Deduction of optimum λ_{exc} and λ_{em}

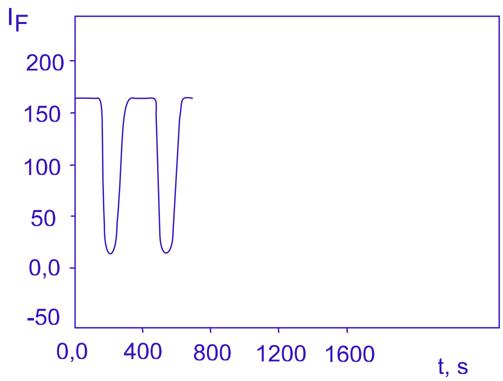

Fig. 3 Typical fiagrams obtained upon the injection of 243 µl of a standard solution of bromate in duplicate (λ_{exc}=339 nm; λ_{em}=421 nm)

higher than 4.3 in order to achieve maximum fluorescence emission. Under these conditions, the length of the reactor was changed from 300 to 1,350 cm at room temperature (23 °C).

The values of ΔI_F are shown in Fig. 4 versus the reactor length (bottom) and reaction time (top). It may be seen that under these experimental conditions the reaction exhibits slow kinetics, the analytical signal increasing with the increase in the reaction time and tending to a constant value for times longer than 344 s. In no case, not even for very long reactors, did dispersion predominate over the increase in the analytical signal.

Influence of temperature With the same experimental conditions as those used before, the reactor length was fixed at 400 cm and the device was thermostatted at different temperatures between 23 and 80 °C. The reaction time was the same for all the experiments (t_R=110 s) and the contact time between the reagent bolus and the thermostatted reactor was also the same because the values of F_R and F_P were constant (98 s). The time of appearance (t_a) was the same for all cases, 120 s. The values of ΔI_F obtained for each temperature are plotted in Fig. 5.

Upon increasing the temperature, the analytical signal increased, due to the increase in the reaction rate, such that for this reaction time a constant value of ΔI_F was reached as from 60 °C. No conclusions could be drawn as regards the stability of the bromation product since it was not fluorescent.

From a practical point of view, it may be inferred that by thermostatting the reactor at temperatures above 40 °C it is possible to accelerate the reaction rate to a sufficient extent to ensure completion of the reaction.

Optimization of the experimental conditions

Once the kinetic characteristics of the chemical reaction were known, a study was made of each of the variables affecting the analytical signal, bearing in mind that it was necessary to differentiate between those affecting the chemical reaction and those that modified the value of the fluorescence emission intensity.

Chemical variables

Using the flow scheme depicted in Fig. 1a, the geometric and hydrodynamic conditions were fixed at the following values: F_R = F_P=0.24 ml min^{-1}; F_A=0.24 ml min^{-1}, R=400 cm; T=60 °C, V_i=243 µl of standard solutions of BrO_3^- at concentrations of 200 and 600 µg l^{-1}, such that the reaction time was constant in all cases, t_R=110 s; the contact time with the thermostatted reactor was constant, t_c=98 s, and the time of appearance was the same, t_a=120 s.

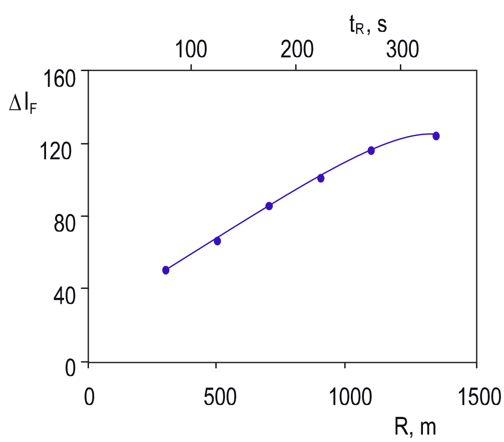

Fig. 4 Determination of BrO_3^- in continuous flow mode by means of the bromation reaction of carbostyiril-124. Kinetic evolution of the reaction at room temperature. C_1: [CARB]=10^{-4} M, [KBr]=6.7×10^{-2} M, [HCl]=0.8 M, F_R=0.24 ml min^{-1}. Channel C_2: Water as carrier, V_i=243 µl of a standard solution of BrO_3^- at a concentration of 200 µg l^{-1}, F_P=0.24 ml min^{-1}. Channel C_3: [NaAc]=2.5 M, F_A=0.24 ml min^{-1}

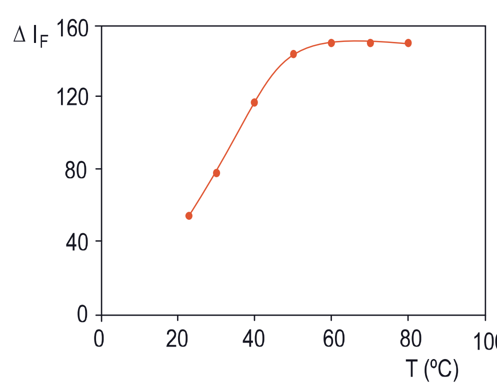

Fig. 5 Influence of temperature on the reaction kinetics. C_1: [CARB]=10^{-4} M, [KBr]=6.7×10^{-2} M, [HCl]=0.8 M, F_R=0.24 ml min^{-1}. Channel C_2: Water as carrier, V_i=243 µl of a standard solution of BrO_3^- at a concentration of 200 µg l^{-1}, F_P=0.24 ml min^{-1}. Channel C_3: [NaAc]=2.5 M, F_A=0.24 ml min^{-1}

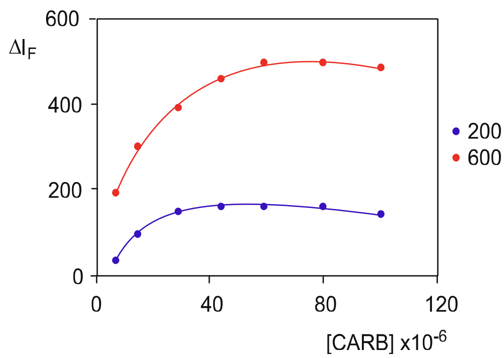

Fig. 6 Influence of concentration of CARB. **a** 600 μg l^{-1} of BrO_3^-, b: 200 μg l^{-1} of BrO_3^-. [KBr]=8.4×10^{-2} M; [NaAc]=2.5 M. $F_R = F_P$= 0.24 ml min^{-1}; F_A=0.24 ml min^{-1}; R=400 cm; T=60 °C; V_i=243 μl

Except when each variable was studied, the following general conditions were imposed for the study: [CARB]= 10^{-4} M; [KBr]=8.4×10^{-2} M; [NaAc]=2.5 M.

Concentration of CARB The CARB concentration was modified between 7.3×10^{-6} M and 10^{-4} M. For each concentration studied, the baseline had a different I_F value, as is logical, but what was being measured was the drop in fluorescence, ΔI_F. The mean ΔI_F values obtained for each concentration of the fluorescent reagent are shown in Fig. 6.

The higher the concentration of CARB, the greater the amount of bromated product formed and the higher ΔI_F, this reaching a value after which the reaction was independent of the concentration of the fluorescent reagent. This constant value was logically different for each concentration of BrO_3^-, being 4.0×10^{-5} M for 200 μg l^{-1} and 5.9×10^{-5} M for 600 μg l^{-1}. Higher CARB concentrations led to a slight decrease in the analytical signal, probably because at those concentrations the organic molecule undergoes a process of self-absorption of the radiation emitted, the quantum yield thus decreasing.

Since the flow rates of the solutions carried by C_1 and C_2 were equal, the real concentrations of CARB at the point of confluence were exactly half (double dilution).

For practical purposes, it may be inferred that up to 200 μg l^{-1} of BrO_3^- the optimum concentration at confluence for the bromation reaction is 2.0×10^{-5} M, although higher concentrations up to 5.0×10^{-5} M generated similar signals. The same was the case for concentrations higher than 200 μg l^{-1} and up to 600 μg l^{-1}; the optimum CARB concentration was 3.0×10^{-5} M, although higher ones, up to 5.0×10^{-5} M, generated similar signals.

Concentration of KBr Once the other variables had been set at the indicated values, the concentration of Br^- in the solution flowing through channel C_1 was modified from 4.0×10^{-3} M to 8.4×10^{-2} M, corresponding to 2.0×10^{-3} M to 4.2×10^{-2} M at the point of confluence where the bromation reaction begins. The results are shown in Fig. 7.

It may be seen that as the concentration of Br^- increased, so did the analytical signal, because it did this at the rate of the reaction with BrO_3^-, a constant value being reached during the reaction time studied.

For concentrations up to 200 μg l^{-1} of BrO_3^-, the analytical signal was constant as from a Br^- concentration of 3.0×10^{-2} in the solution flowing through C_1, corresponding to 1.5×10^{-2} M at the point of confluence, after which the chemical reaction took place. For BrO_3^- concentrations higher than 200 μg l^{-1} (up to 600 μg l^{-1}), a constant analytical signal was obtained as from a slightly higher Br^- value, 4.5×10^{-2} M, corresponding to 2.25×10^{-2} M at the confluence point.

HCl concentration To study the effect of [H^+], the concentration of HCl of the solution flowing through C_1 was modified from 0.05 to 1.2 M, the rest of the variables being kept at the indicated values. The concentration of NaAc, 2.5 M, ensured that at all the HCl values studied the pH of the mixture of solutions at the second confluence point would be higher than 4.3 (a value after which the CARB species showed a constant value of fluorescence emission). For the highest HCl concentration studied, 1.22 M, the pH at the second point of confluence was 5.4.

The values of ΔI_F obtained, as the mean value of three injections, for each [H^+] are plotted in Fig. 8.

It may be seen that the rate of the bromation reaction was independent of the concentration of H^+, a constant value of the analytical signal being reached as from [HCl]= 0.6 M, regardless of the BrO_3^- concentration. The reaction rate increased with [HCl], the analytical signal reaching a constant value as from 0.3 M [HCl] at the first point of confluence.

NaAc concentration To check the effect of the concentration of sodium acetate on the analytical signal after the chemical reaction, it was modified from 0.05 to 3.0 M. Since the concentration of HCl was kept at 0.8 M (0.4 M at

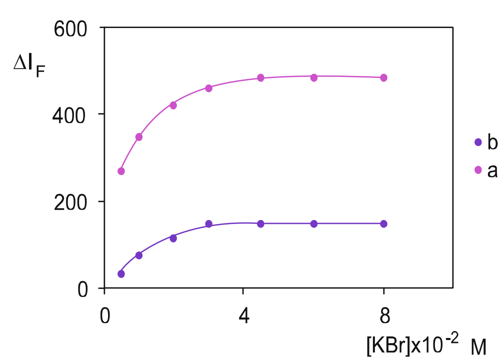

Fig. 7 Influence of concentration of KBr. *a* 600 μg l^{-1} of BrO_3^-, *b* 200 μg l^{-1} of BrO_3. [CARB]=10^{-4} M; [NaAc]=2.5 M. $F_R = F_P$= 0.24 ml min^{-1}; F_A=0.24 ml min^{-1}; R=400 cm; T=60 °C; V_i=243 μl

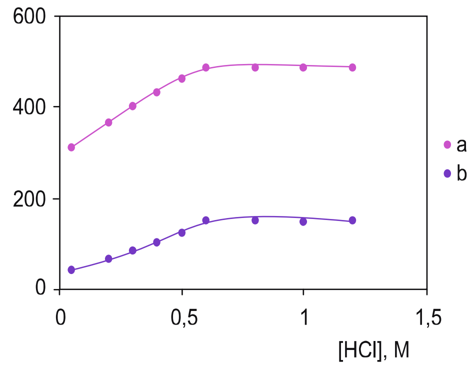

Fig. 8 Influence of concentration of HCl. *a* 600 μg l^{-1} of BrO_3^-, *b* 200 μg l^{-1} of BrO_3^-. [CARB]=10^{-4} M; [NaAc]=2.5 M. F_R = F_P= 0.24 ml min^{-1}; F_A=0.24 ml min^{-1}; R=400 cm; T=60 °C; V_i=243 μl

the first point of confluence), the variations in the analytical signal obtained can only be attributed to the modification in fluorescence emission due to the variation in the concentration of the basic form of CARB. The values of ΔI_F are plotted against the NaAc concentration in Fig. 9.

It may be observed that for values lower than 0.3 M of NaAc the analytical signal was lower than the constant value reached for higher concentrations; this is because the pH at the second point of confluence was lower than the pK_a +1, a zone of predominance of the basic, and fluorescent, form of CARB. Bearing in mind that the concentration of HCl was diluted at the first point of confluence once a value of 0.4 M had been reached, it may be deduced that from the practical point of view the optimum ratio between [Ac] and [K^+] at the second point of confluence should be [Ac^-]/[H^+] >0.7, with which the maximum value of fluorescence emission is ensured for each concentration of BrO_3^-.

Geometric and hydrodynamic variables

Next, a study was made of the geometric and hydrodynamic variables affecting the analytical signal for different reasons. The reaction time is governed by the flow rate from the first point of confluence; that is, the values of F_R and F_P, and the length of the reactor R. Both variables are responsible for determining the time that the reaction bolus is in the thermostatted bath, which is always slightly shorter than the reaction time. Also, the ratio between F_R and F_P controls the dilution of the reagents and, more importantly, the dilution of the sample containing BrO_3^- injected, which affects the sensitivity of the method. Finally, the value of F_A affects the rate of determination (which was not a goal of this study); more importantly, however, it also affects the dilution and hence the sensitivity of the analytical signal.

In light of the above, the study of flow rates was carried out as follows:

A. *Influence of F_A: flow rate of the sodium acetate solution*

The aim of using this solution was to adjust the pH of the other mixed solutions in which the bromation reaction had occurred in order to reach a pH of >4.3 for measurement of the fluorescence of the reagent CARB. However, when at the second point of confluence of the flow scheme depicted in Fig. 1a this solution of sodium acetate was mixed with the solution in which the bromation reaction had already occurred, owing to a dilution effect the concentration of all the products generated decreased, thus affecting the sensitivity of the analytical signal.

With a view to determining the optimum value of F_A in the above flow scheme, the following conditions were fixed: Reagent solution, R: F_R=0.24 ml min^{-1}, [CARB]= 8.0×10^{-5}, [HCl]=1.2 M and [KBr]=8.4×10^{-2} M. Carrier solution, P: F_P=0.24 ml min^{-1}, V_i=243 μl of a solution of BrO_3^- at a concentration of 200 μg l^{-1}. Reactor 400 cm, thermostatted at 60 °C. Under these conditions, and keeping F_R = F_A, the chemical bromation reaction occurred with the optimum values of all the species involved in it.

For the sodium acetate solution, a high concentration (3.0 M) was fixed and its flow rate, F_A was modified from 0.08 ml min^{-1} to 0.48 ml min^{-1}, such that at the second point of confluence it was ensured that [NaAc]/[H^+] >0.7 in all cases, the ratio being smaller for the lowest flow rate, F_A=0.08 ml min^{-1}, at which the concentration ratio was 0.71.

F_A flow rates lower than 0.08 ml min^{-1} (keeping F_R + F_A=0.48 ml min^{-1}) elicited poor mixing at the point of confluence, leading to alterations in the fiagram that affected the precision of the analytical signal. The results are shown in Fig. 10.

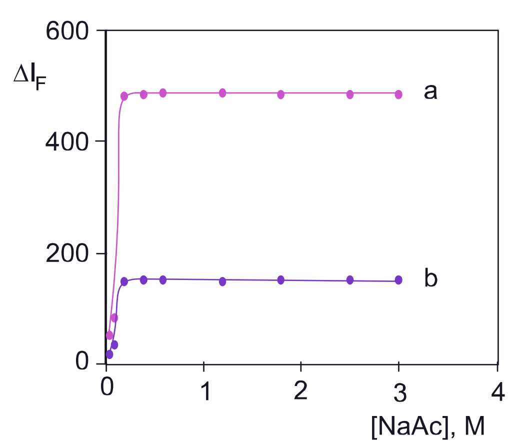

Fig. 9 Influence of concentration of NaAc. *a* 600 μg l^{-1} of BrO_3^-, *b* 200 μg $l^{-1}$of BrO_3^-. [CARB]=10^{-4} M. F_R = F_P=0.24 ml min^{-1}; F_A= 0.24 ml min^{-1}; R=400 cm; T=60 °C; V_i=243 μl

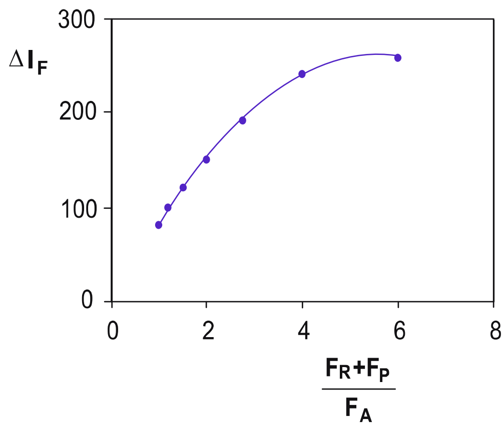

Fig. 10 Influence of flow rate ratios between $F_R + F_P$ and F_A. C_1: [CARB]=10^{-4} M, [KBr]=8.4×10^{-2} M, [HCl]=0.8 M, F_R=0.24 ml min^{-1}. Channel C_2: Water as carrier, V_i=243 µl of a standard solution of BrO_3^- at a concentration of 200 µg l^{-1}, F_P=0.24 ml min^{-1}. Channel C_3: [NaAc]=2.5 M, F_A=0.24 ml min^{-1}

Keeping the value of $F_R + F_P$ constant at 0.48 mL min^{-1} in all the experiments, both the reaction time and the time of contact with the thermostatted reactor remained constant (t_R=98 s, t_c=110 s), such that the changes in the values of ΔI_F can only be attributed to dilution processes of the reagent bolus upon mixing with the solution of sodium acetate. It may be seen that the lower the value of the flow rate F_A and the higher the $F_R + F_P/F_A$ ratio, the higher the value of ΔI_F, because the dilution of the sample injected is lower. From the point of view of analytical sensitivity it was therefore of interest to work with flow rate ratios, $F_R + F_P/F_A$, of the order of 6.

The increase in sensitivity was counteracted by an increase in Δt, the width of the fiagram (FIA signal) at the baseline, as a result of the lower value of F_t, which varied between 0.56 and 0.96 ml min^{-1}. The value of t_a decreased in the same sense as the value of F_A increased, although its variation as from 120 s was difficult to measure with precision since the segment of the Teflon tube joining the second point of confluence with the flow cell was short and the times differed by less than 2 s.

B. *Influence of the flow speed ratio F_P/F_R*

The flow rates, F_P and F_R, governed the bromation reaction time as from the first point of confluence together with the time of contact between the reagent bolus and the thermostatted reactor. Additionally, at the first point of confluence the relationship between F_P and F_R affected the dilution of the standard solution or of the sample injected, and also the dilution of reagents. To study the effect of this relationship on the analytical signal, the reaction time, the contact time, the chemical variables observed in the previous study, the optimum ratio for $F_P + F_R/F_A$, the reaction temperature and the volumes of standard solution injected (243 µl of BrO_3^- at a concentration of 200 µg l^{-1}) were all kept constant and the values of F_P, F_R and F_A were modified.

The value of $F_P + F_R$ was kept at 0.48 ml min^{-1} (the optimum value deduced previously). The value of F_P was varied from 0.22 ml min^{-1} to 0.38 ml min^{-1} and at the same time F_R adopted complementary values between 0.26 and 0.10 respectively. In this range of values of F_P/F_R (0.85 to 3.8) the concentration of CARB, which is the most critical reagent, was kept at the optimum value, 2×10^{-5}–5×10^{-5} M.

The flow rates were controlled with three peristaltic pumps in order to be able to modify F_P, F_R and F_A individually at different values. The values of ΔI_F (λ_{exc}=339 nm, λ_{em}=430 nm) obtained upon injecting 243 µl of a solution of BrO_3^- at 200 µg l^{-1} under the different experimental conditions are plotted in Fig. 11 against the F_P/F_R ratio. It may be seen that the analytical signal ΔI_F increased with the increase in the F_P/F_R ratio because the sample injected underwent a lower dilution as the difference between the values of F_P and F_R increased.

As a practical conclusion, it may be deduced that the highest analytical signal, keeping the time constant at 110 s, can be obtained when F_P has a value of 0.38 ml min^{-1} and F_R=0.10 ml min^{-1} (F_P/F_R=3.8). It is not possible to obtain higher ratios since the CARB reagent would be diluted below the optimum values. In all the experiments the values of t_a and Δt were constant (122 and 180 s respectively).

Influence of injection volume To study the influence of the volume of standard solution or sample injected, using the flow scheme depicted in Fig. 1b the following chemical conditions were fixed: [CARB]=10^{-4} M; [HCl]=1.2 M, [KBr]=8.4×10^{-2} M, [NaAc]=3.0 M. The reactor length was kept at 400 cm, thermostatted at 60 °C, and in cases a standard solution of BrO_3^- at 200 µg l^{-1} was injected. Figure 12 shows the values of ΔI_F obtained for each experiment plotted against the volume injected.

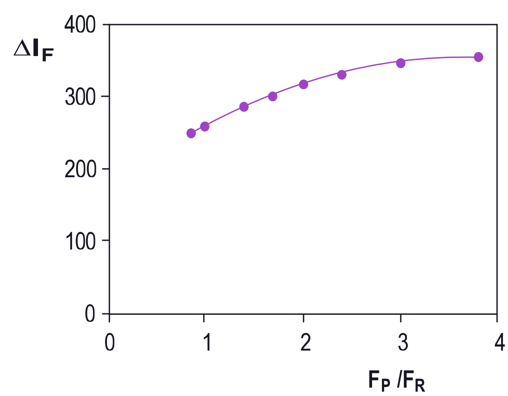

Fig. 11 Influence of flow rate ratio, F_P/F_R keeping $F_P + F_R$=0.48 ml min^{-1} constant and F_A=0.08 ml min^{-1}. C_1: [CARB]=10^{-4} M, [KBr]=8.4×10^{-2} M, [HCl]=0.8 M. Channel C_2: Water as carrier, V_i=243 µl of a standard solution of BrO_3^- at a concentration of 200 µg l^{-1}. Channel C_3: [NaAc]=2.5 M, t_R=110 s, t_C=98 s

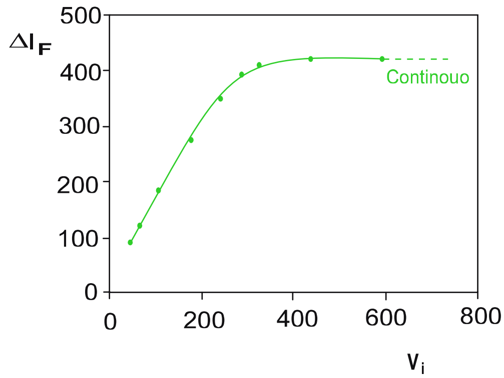

Fig. 12 Influence of injection volume. C_1: [CARB]=10^{-4} M, [KBr]=8.4×10^{-2} M, [HCl]=1.2 M. Channel C_2: Water as carrier, standard solution of BrO_3^- at a concentration of 200 μg l^{-1}. Channel C_3: [NaAc]=3.0 M, $F_R = F_A$=0.10 ml min^{-1}; F_P=0. 38 ml min^{-1}; R=400 cm; T=60 °C

As expected, the larger the volume injected the higher the value of the analytical signal; this is because the dispersion of the bolus injected decreased and therefore the concentration of the analyte in that zone increased. Above 288 μl, the increase began to be less marked, tending to reach the value obtained without dispersion because the transit of the standard solution of BrO_3^- at 300 μg l^{-1} was in continuous mode. From the practical point of view, to conjugate sensitivity and sampling speed, which decreased with the increase in V_i, 288 μl was chosen as a suitable injection volume. In all experiments performed, t_R=110 s, t_c=98 s, t_a=120 s.

Influence of bromate concentration analytical calibration Once the optimum chemical, geometric and hydrodynamic conditions had been selected, a study was made of the relationship between the concentration of BrO_3^- and the analytical signal ΔI_F. In the flow scheme shown in Fig. 1b, the following conditions were imposed: C_1: F_P=0.38 ml min^{-1}, V_i=288 μl, C_2: F_R=0.10 ml min^{-1}, [CARB]=10^{-4} M, [HCl]=1.2 M, [KBr]=8.4×10^{-2} M, C_3: F_A=0.10 ml min^{-1}, [NaAc]=3.0 M, R=400 cm, thermostatting temperature = 60 °C. Standard solutions of bromate were prepared at concentrations ranging between 4 and 200 μg l^{-1}; these were injected into the flow system in triplicate (V_i=288 μl).

From the results obtained it was found that the values of ΔI_F (λ_{exc}=339 nm, λ_{em}=430 nm) as a function of the concentration of BrO_3^- fitted a straight line with the following equation:

$$\Delta I_F=(-0.4\pm2.3)+(2.08\pm0.03)\left[BrO_3^-\right],\ \mu g l^{-1} R^2=0.9998,$$

for a level of confidence of 95%.

Under these conditions, the concentration of the limit of detection calculated by the simplified expression C_L= $3X_b^{max}/b$ (X_b^{max}=0.6) was 0.9 μg l^{-1}. These observations indicate that method of bromate determination is clearly more sensitive than the spectrophotometric methods described, and with a broad range of linearity, up to 200 μg l^{-1}. This allows the measurement of BrO_3^- concentrations at around current parametric levels and those to be established in the future (recall that Japan and the USA recommend 5 μg l^{-1}).

Precision of the method To study the precision of the method, 12 standard solutions of BrO_3^- (n=12) containing 5 μg l^{-1} and another 12 at concentrations higher than 30.0 μg l^{-1} were prepared and injected (V_i=288 μl) into the flow system After the individual values of ΔI_F had been measured and after the corresponding statistical treatments, values of S_R=3.2% and S_R=2.6% respectively were obtained for the relative standard deviation. The determination rate was between 10 and 12 determinations per hour.

Study of interferences

The possible cationic and anionic interferents most frequently found in water were studied, together with other less frequent ones of interest, at varying concentrations. In this part of the work, standard solutions of BrO_3^- at 20 μg l^{-1} with and without the interferent under scrutiny were injected under the experimental conditions found in the calibration for BrO_3^-. The results are shown in Table 1.

Table 1 Study of interferences

Interferent	Does not interfere up to a concentration of ≤	Parametric value
Cationic interferents		
NH_4^+	5 mg l^{-1}	05 mg l^{-1}
Na^+	400 mg l^{-1}	200 mg l^{-1}
Cu^{2+}	5 mg l^{-1}	2 mg l^{-1}
Ca^{2+}	400 mg l^{-1}	–
Mg^{2+}	300 mg l^{-1}	–
Zn^{2+}	8 mg l^{-1}	
Al^{3+}	350 μg l^{-1}	200 μg l^{-1}
Mn^{2+}	230 μg l^{-1}	50 μg l^{-1}
Cd^{2+}	250 μg l^{-1}	5 μg l^{-1}
Fe^{3+}		
Anionic inteferents		
F^-	2.6 mg l^{-1}	1.5 mg l^{-1}
Cl^-	350 mg l^{-1}	250 mg l^{-1}
Br^-	80 mg l^{-1}	–
NO_3^-	100 mg l^{-1}	50 mg l^{-1}
ClO_3^-	2 mg l^{-1}	–
SO_4^{2-}	400 mg l^{-1}	250 mg l^{-1}
PO_4^{3-}	16 mg l^{-1}	–
ClO_2^-	5 μg l^{-1}	

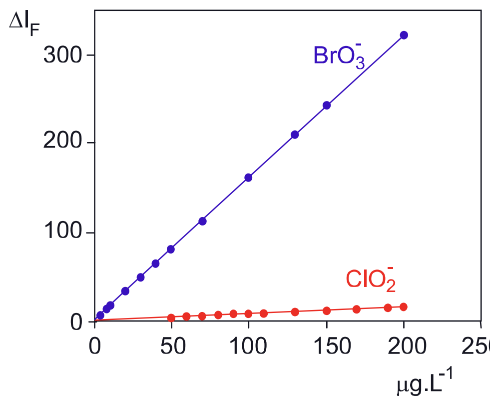

Fig. 13 Analytical calibration for BrO_3^- at 40 °C and ClO_2^- as interferent at the same temperature. C_1: Water as carrier. Channel C_2: [CARB]=10^{-4} M, [KBr]=8.4×10^{-2} M, [HCl]=1.2 M. Channel C_3: [NaAc]=3.0 M. F_P=0.38 ml min^{-1}, F_R = F_A=0.10 ml min^{-1}, V_i= 288 µl, R=400 cm

Keeping constant the criterion of considering as an interferent any analyte that might modify the analytical signal of BrO_3^- by 5%, of all the possible interferents analyzed only ClO_2^- was found to alter the analytical signal—under the experimental condition used—by more than 5 µg l^{-1}.

ClO_2^- reacts with Br^- in acid medium, generating bromine, which then reacts with CARB, like BrO_3^-. However, in light of the analytical signals generated by both analytes for the same concentration, that of ClO_2^- being lower, it appears that in terms of speed the reaction kinetics is different.

To check this, 288 µl of standard solutions of ClO_2^- was injected into the flow scheme shown in Fig. 1b under the experimental conditions used for the calibration for BrO_3^- at concentrations between 10 and 200 µg L −1. The values of ΔIF obtained were plotted against the concentration of ClO_2^- and it was observed that the analytical signal, ΔIF, fitted a straight line with an equation of:

$$\Delta I_F=(1.0\pm0.6)+(0.397\pm0.005)\left[ClO_2^-\right],\ \mu g l^{-1}$$

for a confidence level of 95% and a linear regression coefficient R^2 of 0.999.

The slope of that line is clearly lower than that obtained under the same conditions for BrO_3^- (0.397 versus 2.08), pointing to a lower reaction rate for the case of ClO_2^-.

Regardless of the fact that the CARB bromation reaction is of analytical use for the determination of ClO_2^- in aqueous systems where there is no BrO_3^-, in order to resolve its interference in the bromate determination method the analytical measurements (signal) generated by both analytes were tested under other kinetic conditions.

The best results were obtained simple by changing the thermostatting temperature of the 400-cm reactor, keeping the other variables constant at the indicated values.

The values of ΔI_F obtained upon thermostatting the reactor at 40 °C and injecting standard solutions of BrO_3^- and ClO_2^- at different concentrations are shown in Fig. 13, where—plotted against concentration—they can be seen to fit straight lines with equations of:

$$BrO_3^-: \Delta I_F=(0.2\pm0.2)+(1.617\pm0.002)\left[BrO_3^-\right],\ \mu g l^{-1}$$

for a confidence level of 95%.

The range of linearity for both analytical signals was kept up to 200 µg l^{-1}, but the slope of the calibration line decreased with respect to that obtained at 60 °C.

The method for bromate determination was less sensitive at 40 °C (C_L=2.2 µg l^{-1}) but sufficient for application in the neighborhood of the parametric values (10–20 µg l^{-1}); also, at that temperature the interference by ClO_2^- decreased.

It may be concluded that ClO_2^- at 40 °C interferes in the determination of BrO_3^- in water subjected to purification when the concentration of the interferent higher than 50 µg l^{-1}.

In order to validate the method for the determination of BrO_3^- by means of the bromation of CARB, well water (Robledo Hermoso, Salamanca, Spain) and mineral water (Aquabona) samples were spiked with standard solutions of the analyte at a final concentration ranging between 10 and 50 BrO_3^- and they were determined under the calibration conditions at 60 °C.

The results obtained for both matrices are shown in Table 2; they were obtained after injecting each spiked solution in triplicate. It may be seen that for a level of confidence of 95% there is no significant difference between the BrO_3^- added and that found.

Conclusions

This work describes a new sensitive and selective procedure for the determination of bromate that allows online measurement of trace levels of this compound in water with no need for previous separation or preconcentration steps.

The method is based on the bromation reaction of CARB in a flow system that permits kinetic control of the reaction with fluorimetric detection The procedure, which is easy to

Table 2 Determination of BrO_3^-, in continuous flow mode by means of the bromation reaction of carbostyril-124

$[BrO_3^-]$ added (µg l^{-1})	$[BrO_3^-]$ found (µg l^{-1})	
	Well water	Mineral water
10	11±3	12±3
20	21±3	20±3
30	28±3	29±3
40	39±3	38±3
50	52±3	48±3

Validation of method

automate and simple, allows determination of the analyte across a broad concentration range (4–200 μg l^{-1}) with a limit of detection of 0.9 μg l^{-1}, such that it is perfectly adaptable to the needs demanded by current legislation and those foreseen for developed countries.

References

1. Weinberg HS, Yamada H, Joice RJ (1998) New, sensitive and selective method for determining sub-mg/l levels of bromate in drinking water. J Chromatogr A 804:137–142
2. van Staden JF, Mulaudzi LV, Stefan RI (2004) Spectrophotometric determination of bromate by sequential injection analysis. Talanta 64:1196–1202
3. Delker D, Hatch G, Allen J, Crissman B, George M, Meter D, Kilburn S, Moore T, Nelson G, Roop B, Slade R, Swank A, Ward W, De Angelo A (2006) Molecular biomarkers of oxidative stress associated with bromate carcinogenicity. Toxicology 221:158–165
4. Kurokowa Y, Takamura N, Matsuoka C, Imazawa T, Matsushima Y, Onodera H, Hayashi Y (1987) Comparative studies on lipid peroxidation in the kidney of rats, mice, and hamsters and on the effect of cysteine, glutathione, and diethyl maleate treatment on mortality and nephrotoxicity after administration of potassium bromate. J Am Coll Toxicol 6:489–501
5. Kurokawa Y, Maekawa A, Takahashi M, Hayashi Y (1990) Toxicity and carcinogenicity of potassium bromate—a new renal carcinogen. Environ Health Perspect 87:309–335
6. IARC (1986) IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Some naturally occurring and synthetic food components, furocoumarins and ultraviolet radiation. IARC Publication No. 40, World Health Organization/ IARC, Lyon, France, pp 207–220
7. Crofton KM (2006) Bromate: concern for developmental neurotoxicity? Toxicology 221:212–216
8. Council Directive 98/83/EC (1998) Official Journal of the European Communities Legislation, 1998, p 32, November 3rd
9. U.S. Environmental Protection Agency, National Primary Drinking Water Regulations: Disinfectants and Disinfection Byproducts; Final Rule, Fed. Reg., 63, No. 241 (1998) 69390
10. Ingrand V, Guinamant JL, Bruchet A, Brosse C, Noij ThHM, Brandt A, Sacher F, McLeod C, Elwaer AR, Croué JP, Quevauviller Ph (2002) Determination of bromate in drinking water: development of laboratory and field methods. Trends Anal Chem 21(1):1–12
11. EPA method 300.0 (1991) EPA/600/R93/100
12. EPA method 300.1 (1997) EPA/600/R98/188
13. ISO/DIS (1998) 15061
14. Wagner HP, Pepich BV, Hautman DP, Munich DJ (1999) Analysis of 500-ng/l levels of bromate in drinking water by direct-injection suppressed ion chromatography coupled with a single, pneumatically delivered post-column reagent. J Chromatogr A 850:119–129
15. Wagner HP, Pepich BV, Hautman DP, Munich DJ (2000) Eliminating the chlorite interference in US Environmental Protection Agency Method 317.0 permits analysis of trace bromate levels in all drinking water matrices. J Chromatogr A 882:309–319
16. Wagner HP, Pepich BV, Hautman DP, Munich DJ (2000) Performance evaluation of a method for the determination of bromate in drinking water by ion chromatography (EPA Method 317.0) and validation of EPA Method 324.0. J Chromatogr A 884:201–210
17. Wagner HP, Pepich BV, Hautman DP, Munich DJ (2002) US Environmental Protection Agency Method 326.0, a new method for monitoring inorganic oxyhalides and optimization of the postcolumn derivatization for the selective determination of trace levels of bromate. J Chromatogr A 956:93–101
18. Takayanagi T, Ishida M, Mbuna J, Driouich R, Motomizu S (2006) Determination of bromate ion in drinking water by capillary zone electrophoresis with direct photometric detection. J Chromatogr A 1128:298–302
19. Yamanaka M, Sakai T, Kumagai H, Inoue Y (1997) Specific determination of bromate and iodate in ozonized water by ion chromatography with postcolumn derivatization and inductively-coupled plasma mass spectrometry. J Chromatogr A 789:259–265
20. Divjack B, Novic M, Goessler W (1999) Determination of bromide, bromate and other anions with ion chromatography and an inductively coupled plasma mass spectrometer as element-specific detector. J Chromatogr A 862:39–47
21. Dudoit A, Pergantis SA (2001) Ion chromatography in series with conductivity detection and inductively coupled plasma-mass spectrometry for the determination of nine alojen, metalloid and non-metal species in drinking water. J Anal At Spectrom 16:575–580
22. Elwaer AR, McLeod CW, Thompson KC (2000) On-line separation and determination of bromate in drinking waters using flow injection ICP mass spectrometry. Anal Chem 72:5725–5730
23. Valsecchi S, Isernia A, Polesello S, Cavalli S (1999) Ion chromatography determination of trace level bromate by large volume injection with conductivity and spectrophotometric detection after post column derivatisation. J Chromatogr A 864:263–270
24. Achilli M, Romele L (1999) Ion chromatographic determination of bromate in drinking water by post-column reaction with fuchsin. J Chromatogr A 847:271–277
25. Magnuson ML (1998) Determination of bromate at parts-per-trillion levels by gas chromatography–mass spectrometry with negative chemical ionization. Anal Chim Acta 377:53–60
26. Silva JCGED, Dias JRM, Magalhaes JMCS (2001) Factorial analysis of a chemiluminescence system for bromate detection in water. Anal Chim Acta 450:175–184
27. Diemer J, Heumann KG (1997) Bromide/bromate speciation by NTI-IDMS and ICP-MS coupled ion exchange chromatography. Fresenius J Anal Chem 357:74–79
28. Creed JT, Brockhoff C (1999) Isotope dilution analysis of bromate in drinking water matrixes by ion chromatography with inductively coupled plasma mass spectrometric detection. Anal Chem 71:722–726
29. Uraisin K, Takayanagi T, Nacapricha D, Motomizu S (2006) Novel oxidation reaction of prochlorperazine with bromate in the presence of synergistic activators and its application to trace determination by flow injection/spectrophotometric method. Anal Chim Acta 580:68–74