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DIFFICULTIES WITH THE CHLORAMINE-T–PHENOL RED METHOD FOR BROMIDE DETERMINATION

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Summary—The chloramine-T–Phenol Red procedure affords a potentially very sensitive photometric method for the determination of bromide. However, serious problems (poor precision and high reagent blanks) have been encountered in trials which followed exactly the published procedure. The reason for these difficulties was found to be the high ratio of chloramine-T to Phenol Red which was used. In all previously reported applications of this method a ratio in excess of 4 was employed, and these publications also mentioned problems with the reproducibility. In the current work the use of a reagent ratio of 1.5 was found to overcome these difficulties and yield a robust method of excellent precision. At the same time, the previously reported strong interferences by chloride and ammonia were also effectively eliminated.

The measurement of bromide at low concentrations is of great importance in the food and drug industries and for assessing the effects of chlorination of seawater used as a coolant in thermal power stations. However, the impetus for this study was provided by the selection of this anion as a tracer for measuring the hydrodynamic properties (flow velocities and dispersion coefficients) of streams. Bromide is almost ideal for this purpose since very little is lost from solution, either by adsorption on the bed sediments or by reaction with other components in the flowing waters.[1]

Since the tracer (in the form of lithium bromide) was to be released into the water as a short duration pulse, the peak concentration was expected to vary from decimolar to micromolar as the pulse was broadened by dispersion along the section of stream being studied. It was therefore essential to have an analytical method which was capable of detecting bromide at the micromolar level. The requirement for high-quality hydrodynamic data also meant that the chosen method had to yield highly accurate and precise measurements of concentration.

A survey of the literature revealed that a colorimetric method based on the conversion of Phenol Red (PR) into Bromophenol Blue (BPB) satisfied the requirement for high sensitivity. The reaction of hypobromite with PR to produce BPB was first suggested in 1935 as a method for the determination of micro quantities of bromide in solution.[2] The bromide was oxidized to hypobromite by the addition of sodium hypochlorite. Hypochlorite was subsequently replaced by chloramine-T (CT) as the oxidizing agent. The overall reaction is summarized in equation (1):

$$PR + 4Br^- + 4H^+ + 4CT(O) \rightarrow BPB + 4CT(R) \quad (1)$$

where (O) = oxidized form, (R) = reduced form.

Thiosulphate is added in a final step to decompose the excess of CT, which would otherwise oxidatively bleach the BPB.

Although the procedure as given in "*Standard Methods for the Examination of Water and Wastewater*" appeared to be quite straightforward,[3] severe problems were encountered with both lack of reproducibility for the test samples, and high reagent blanks in an initial attempt to use it. The discovery of the reasons for this behaviour forms the basis of this paper. As a result of this work a simple modification to the procedure was made which yields a much more robust and precise technique for the determination of bromide.

EXPERIMENTAL

The procedure outlined in Standard Methods was followed,[3] with the reagent additions scaled to a sample volume of 10 ml. The final CT concentration in the polyethylene vials used as reaction vessels was 38.6μM, which is a quarter of that recommended.[3] The reagent volumes and concentrations used are compared with those in the Standard Method, in Table 1. For those determinations with a CT concentration of 386μM, the reaction was quenched with thiosulphate exactly 3.5 min after the oxidizing agent was added.

The absorbances of the test solutions were measured at 590 nm in a 10-mm path-length cell with a Varian 635 spectrophotometer. A Dionex Model 10 ion chromatograph containing S2 separator columns was also used to analyse the water samples collected in the field. The relatively high level of sulphate (1.5mM) necessitated the use of a modified eluent (4.5mM sodium carbonate, 2.0mM sodium hydroxide, 10% methanol) to effect complete separation of the bromide and sulphate peaks. The analyses were done at an operating pressure of 580 psig, with a conductivity detector.

RESULTS AND DISCUSSION

Initial tests of the Standard Method[3] by the author yielded very high and non-reproducible absorbance

Table 1. Conditions used for bromide determination*

	Standard method[3]	Modified method (this work)
Sample, *ml*	50	10
Acetate buffer, *ml*	2	0.5
Phenol Red, *ml*	2	0.5
Chloramine-T, *ml*	0.5 (5 mg/ml solution)	0.5 (0.25 mg/ml solution)
Thiosulphate, *ml*	0.5	0.13
Total volume, *ml*	55	11.63
Reaction time, *min*	20	20

*Concentrations of reagent solutions are identical to those for the standard method except where specified.

values (0.38–0.5) for the reagent blank solutions. Contamination of the reagents was initially suspected, so the CT and PR were recrystallized. High-purity acetic acid and sodium acetate were used to prepare the buffer solution. However, the use of these purified reagents did not solve the problem.

Further investigation revealed that the CT concentration was the critical variable. The maximum reagent blank absorbances (at 590 nm) obtained for CT concentrations of 39, 78 and 195μM were 0.026, 0.15 and 0.35, respectively. It was also observed that the rates of colour production and subsequent decay in the reagent blank were very dependent on the CT concentration, the rates being much faster with higher concentrations of this reagent. Curve 2 in Fig. 1 illustrates the absorbance changes occurring at 590 nm when the initial CT concentration was 386 μM ($[PR]_0 = 26\mu M$). When the reaction was repeated in the presence of 25μM Br^-, curve 4 was obtained. The initial colour development is very rapid but the chromophore is then bleached by a slower secondary reaction with hypochlorite generated by decomposition of the excess of CT present. It is clear that under these conditions the timing of the addition of the quenching reagent, thiosulphate, is extremely critical.

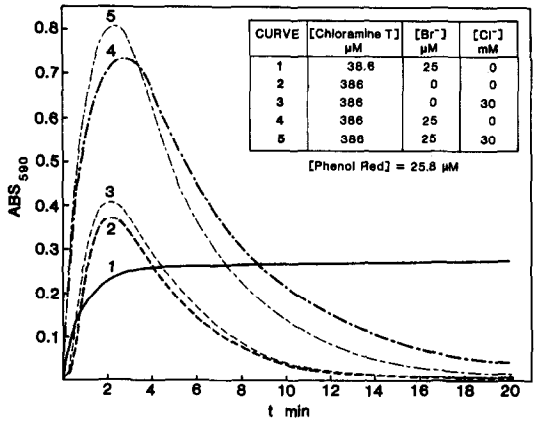

Fig. 1. Effect of CT and chloride concentrations on the kinetics of colour production and decay (measured at 590 nm).

It was suspected that the colour of the blank (curve 2) was due to the production of Chlorophenol Blue (CPB), since the visible spectrum of this compound is very similar to that of BPB. This assertion was confirmed by quenching the reaction after 2 min and separating the reaction products by chromatography on Sephadex DEAE anion-exchange resin at pH 4.5. Under these conditions CPB would be in its deprotonated blue form whereas Phenol Red and Dichlorophenol Red would be protonated and hence yellow and yellow-orange, respectively. This experiment showed that CPB was the major product.

Since the minimum reagent blank absorbance was obtained with a CT concentration of 39μM, it was decided to test the performance of the method under these conditions. The absorbance of the reagent blank was found to be only 0.006 after 10 min and curve 1 in Fig. 1 shows that the colour due to BPB is very stable once produced. Accordingly, the reagent concentrations used to produce curve 1 were chosen as the basis for further application of this method. The calibration data (corrected for reagent blank) obtained under these conditions are shown in Fig. 2, and represent the average of 13 determinations over a period of 40 days by two operators. Clearly the reproducibility is excellent. The modified conditions, with a CT to PR ratio of 1.5, have thus yielded a robust procedure. It should be noted that all previous applications of this method[3-5] have used a ratio in excess of 4.

From the data in Fig. 2 it is apparent that approximately 40μM Br^- is the maximum concentration that can be accurately determined. Consideration of equation (1) shows that this upper limit is imposed by the stoichiometry of the reaction. Thus all of the added CT would be consumed by an equimolar concentration of bromide.

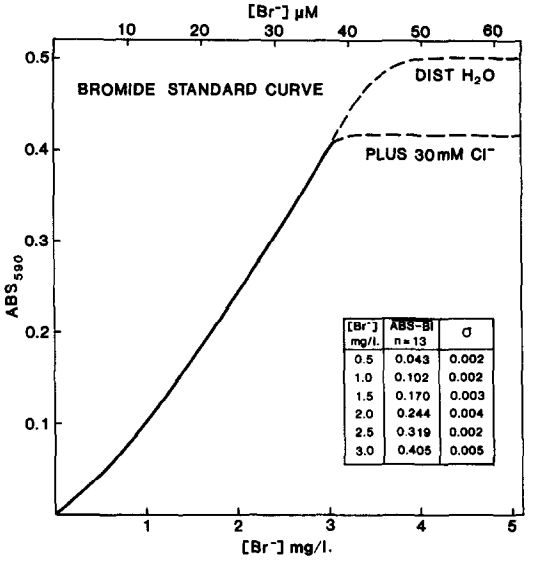

Fig. 2. Standard curve obtained with a CT concentration of 38.6 μM.

Effect of interfering substances

It has been reported that the CT/PR method suffers from a number of significant chemical interferences. Organic compounds, chloride, ammonia, and bicarbonate have each been shown to cause problems with the Standard Method and its published variants.[3-5] The effect of the last of these is easily controlled by ensuring that the acetic acid/acetate buffer used has sufficient capacity to neutralize any bicarbonate present and give a final pH of 4.6 for the sample plus buffer.[4] Consequently, this particular aspect will not be further discussed.

The other interferents are, however, much more troublesome since they react either directly or indirectly with CT, bromide or PR. Chloride can cause a positive interference by reacting to produce Chlorophenol Blue,[4] and organic compounds can produce positive or negative interference, depending on the stability and/or absorption maximum of the brominated product.[5] The presence of ammonia has been shown to cause a serious negative interference.[4]

The extent of interference produced by each of these species will depend on how rapidly they undergo reaction relative to the rate of production of BPB. If interference is observed for a particular set of conditions, it might be possible to reduce the rate of the offending reaction sufficiently to eliminate the interference, by altering the reagent concentrations. In all previous applications of the CT/PR method in which the extent of interference by the species listed above has been reported, a CT to PR ratio in excess of 4 has been used. Since a reduction of the ratio to 1.5 was found to improve the stability and precision of the colour considerably, it was thought that this approach might also be of benefit in the control of chemical interferences. Thus the response of the modified method to the presence of organic compounds, chloride and ammonia has been examined in detail.

Organic compounds. The analytical work in this laboratory is focused on the measurement of the concentrations of compounds in freshwater surface streams in which the organic matter is contributed primarily from natural sources. Consequently, the organics-containing solution used in this study was obtained by extracting a sample of sediment collected from the bed of a dry stream located in a relatively pristine area. The main source of organic material was leaf litter that originated from the grasses and species of eucalypt trees which bordered the stream.

Calibration curves prepared with standards with and without the organics-containing extract present are shown in Fig. 3. The total organic carbon concentration was only 1.3 mg/l., yet this was sufficient to produce a serious negative interference. It is likely that this interference arises from competition between activated aromatic centres in the organic molecules and PR as reactive substrates for hypobromite. Since it is highly improbable that the extracted organic

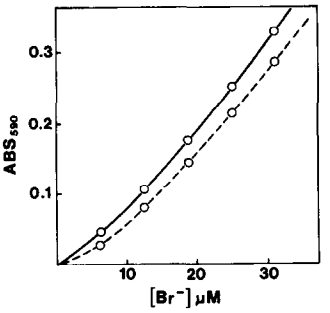

Fig. 3. Depression of standard curve caused by dissolved organic carbon (OC). O—O, [OC] = 0 mg/l.; O----O, [OC] = 1.5 mg/l.

molecules will have an arrangement of aromatic rings similar to that in PR, it is unlikely that the resulting brominated species will have absorbance maxima near 590 nm. Other work has shown that Rhodamine WT (a fluorescent red dye used as a water tracer) also produces a negative interference in the CT/PR method.[6] In this case two atoms of bromide are incorporated into the parent dye molecule. However, the brominated product is not stable and is rapidly bleached. It follows from this that the interference produced by natural and man-made organic compounds in the PR method will generally be negative.

In the current case the organic interference could be compensated for by preparing the calibration curve with solutions containing the same level of organic matter as that in the samples (*i.e.*, organic matrix-matched standards were used). This approach was feasible under these circumstances since there were a large number of samples that contained the same concentration and type of organic matter.

However, when the organic carbon concentration varies widely between samples it would be necessary either to use the method of standard additions or to pretreat the samples to remove the organic material. The use, in a continuous-flow analyser, of an in-line column of macroreticular resin for the latter purpose has been described previously.[5]

Chloride. It has been reported that the CT/PR method suffers from significant interference by chloride.[4] Since the water of the stream to be monitored contained 30mM chloride, it was essential to establish the extent to which this would affect the determination of bromide in the samples collected. Figure 4 shows the effect of 0–50mM chloride on the absorbances obtained for solutions containing 0 or 25μM bromide and 38.6 or 386μM CT. It is obvious from curves 1 and 2 that there is no significant contribution from chloride below 30mM when 38.6μM CT is used and that at above 30mM chloride the increase in absorbance is only very small. It should be noted that 30mM chloride does slightly reduce the maximum concentration of bromide that can be determinated before the plateau in the standard curve is reached (see Fig. 2), presumably by competitive reaction with the CT.

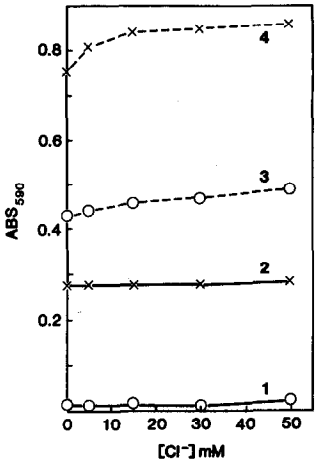

Fig. 4. Effect of CT concentration on extent of chloride interference. Curve (1) [CT] = 38.6μM; [Br] = 0μM; (2) [CT] = 38.6μM; [Br] = 25μM; (3) [CT] = 386μM; [Br] = 0 μM; (4) [CT] = 386μM; [Br] = 25μM.

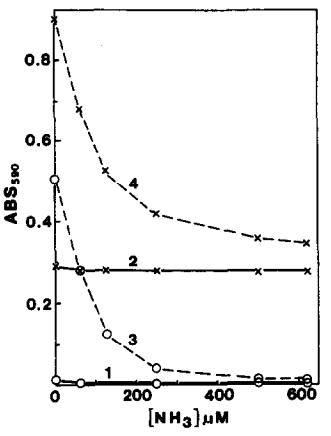

Fig. 5. Effect of CT concentration on extent of interference by ammonia. Curve (1) [CT] = 38.6μM, [Br] = 0μM; (2) [CT] = 38.6μM, [Br] = 25μM; (3) [CT] = 386μM, [Br] = 0μM; (4) [CT] = 386μM, [Br] = 25μM.

However, in marked contrast to this, there is a substantial dependence of absorbance on chloride level in the presence of 386μM CT (curves 3 and 4). Moreover, nowhere are the reagent blank and test solution curves (3 and 4, respectively) parallel. Hence unless the standards contain exactly the same concentration of chloride as the samples, incorrect values will be obtained for the amounts of bromide in the unknowns. The accurate analysis of solutions varying in chloride concentration would therefore be very time-consuming.

A field method for the determination of bromide, which uses peroxymonosulphate (oxone) as the oxidizing agent instead of CT, has been published.[7] Unfortunately, however, strong interference from chloride is still observed. Presumably, the concentration of oxone is high enough for appreciable levels of hypochlorite to be generated by the oxidation of chloride. This species will then react with the PR to produce CPB. The results from the current study suggest that reduction of the oxone to PR ratio might eliminate this problem.

Ammonia. Ammonia has been found to exert a negative interference at levels as low as 3μM (0.05 mg/l.) in the spectrophotometric determination of bromide.[4] The presence of 30μM ammonia in a solution containing 19μM bromide resulted in a depression of colour development such that the bromide concentration found was only 8.5μM.[4] In view of the success of the lower CT to PR ratio in reducing the interference of chloride the effect of the modified conditions on ammonia interference was tested. Figure 5 shows the influence of 0–622μM ammonia on the absorbances for solutions containing 0 or 25μM bromide and 38.6 or 386μM CT. At pH 4.6, all the added ammonia will be present as the ammonium ion.

There is an enormous difference in the results for the two levels of CT. At the lower concentration the absorbances of both the blank and test solutions are essentially independent of the ammonia concentrations. Consequently ammonia does not interfere significantly under these conditions. However, in the presence of 386μM CT the absorbance is a steeply decreasing function of the ammonia level. If the concentration of bromide in a test solution containing ammonia were determined under these conditions, with a set of ammonia-free standards, then a negative interference would be observed, because the reagent blank used in the calculations would be too high. For example, consider the case of a 25μM bromide solution (Fig. 5). If the blank and test solutions contained no ammonia then the blank-corrected absorbance would be 0.393. However, if the ammonia concentration in the test solution were 50μM then the corrected absorbance would be 0.221. Thus the calculated concentration of bromide would be almost 50% low. This example shows that the use of an inappropriate blank value was the reason why previous workers found a negative interference by ammonia.

Making the ammonia concentration in all the standards and samples in a batch at least 600μM would, of course, overcome the problem if a high CT concentration were used. Under these conditions the dependence of the absorbance on the ammonia level would be greatly reduced and the test–blank differential would approach its assymptotic limit. However, there is no need to resort to this procedure since the effect of ammonia is eliminated by using the much lower CT concentration (38.6μM) recommended in this paper. It is also not necessary to pretreat the samples by ion-exchange to remove the ammonia prior to analysis, as has been done previously.

Hydroxylamine. Hydroxylamine has been reported[8] to occur in natural waters under certain conditions, so its possible effect on the method was examined. It proved to be a potent inhibitor of

Bromophenol Blue formation, since it reacts stoichiometrically with chloramine-T, and a level of only 40μM will completely suppress the colour development in the procedure described above. The presence of up to 100μM levels of hydroxylamine has been reported in Ethiopian rivers draining marshlands.[8] Steps should therefore be taken to eliminate this potential interference from suspect natural water samples. Hydroxylamine could also enter rivers since this compound is used as a corrosion inhibitor in boiler water circuits. Work is currently under way in this laboratory to develop a method for decomposing hydroxylamine that is compatible with the chemistry of the spectrophotometric method for bromide.

Comparison with ion-chromatography

The excellent reproducibility of the calibration curve obtained by the modified method is shown in Fig. 2. Duplicate analysis of six different samples obtained from the test stream yielded a value of $29.0 \pm 0.5\mu M$ for the background concentration of bromide. Eleven samples collected on another day gave a value of $29.7 \pm 0.8\mu M$. Suppressed ion-chromatography was also used to analyse eight of these samples to provide a check on the spectrophotometric method. The result obtained was $28 \pm 3\mu M$. Quite clearly the precision of the ion-chromatography was much poorer than that of the spectrophotometric technique. Moreover, ion-chromatography takes at least ten times as much time as the CT/PR method to analyse the same number of samples.

CONCLUSIONS

The use of a chloramine-T to Phenol Red ratio of 1.5 overcomes many of the problems reported in connection with the standard spectrophotometric method for the determination of bromide. The modified procedure can give good precision ($\pm 1.7\%$ at a level of 30μM, which is at least three times better than could be attained with ion-chromatography) and accuracy. The interferences by chloride and ammonia have been effectively eliminated and the timing of addition of thiosulphate is no longer critical.

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