A Colorimetric Microwell Method for Determining Bromide Concentrations in Tracer Studies

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ABSTRACT:

Bromide (Br^-) is commonly used as a tracer in studies of water and chemical transport in soil and rock because it is relatively nonreactive with soil and rock constituents and because of its low environmental background concentrations.

Based upon a largely ignored modification of the standard colorimetric method for determining bromide using phenol red and chloramine-T, we correct an internal error and recast the technique for use with 96-well microplates. Furthermore, the addition of thiosulfate to quench the undesirable chlorination reaction as previously published is shown to be unnecessary and even detrimental following the use of ammonium to produce chloramine from excess chlorine species. By manipulating sample size and concentrations of phenol red and chloramine-T, the concentration range can be expanded from 12 mg L^{-1} Br^- to much as 300 mg L^{-1} Br^-.

Introduction:

Of the several methods available for bromide analysis, ion chromatography (IC) and ion-specific electrodes (ISE) are probably the most familiar for tracer purposes among soil scientists. However, a colorimetric method for analyzing water and wastewater has long been regarded as a standard method jointly by the Am. Public Health Assoc., Am. Water Works Assoc., and the Water Pollution Control Fedn. This standard colorimetric method, virtually unchanged since Stenger and Kolthoff (1935), employs the bromination reaction of phenol red (PR) in the presence of the oxidant chloramine-T (CT), forming bromophenol blue as a product, with the reaction quenched by addition of sodium thiosulfate (TS) before reading absorbance at 590 nm. Chloride is the major chemical interference with this standard method because of a side reaction chlorinating the phenol red, producing a product similar in color to its brominated analog. In a largely ignored paper, Jones (1993) showed that addition of 0.4 mM NH_4 to the phenol red reagent caused the free chlorine to form chloroamine and thereby prevented the undesirable chlorination reaction.

It was our objective to determine the utility of the APHA (1998) standard method as modified by Jones (1993) for column studies and soil lysimeter samples. In the process, we resolved an internal error in Jones' description of the method, scaled the method to microwells on microplates instead of 50-mL volumetric flasks, and determined that the addition of sodium thiosulfate to quench the bromination reaction was contraindicated by the modification made by Jones.

Materials and Methods:

Apparatus:

- flat-bottomed, polystyrene, assay plates (96 350-µL wells)
- 12-channel, 20 to 300-µL electronic pipette
- MRX microplate reader with a 590-nm filter
- PC

Materials and Methods (continued):

Procedure:

Into each microwell, a volume of 300 µL of 0 – 12 mg L^{-1} Br standards or samples is pipetted. To the microwells, 22.5 µL phenol red+NH_4^+ (0.034 mM PR and 0.026 mM NH_4OAc) and 22.5 µL chloramine-T (0.010 mM) are added. The microplates are gently shaken and read at 590 nm after 5 to 15 min for color development. Typically, at least two calibration curves are run per plate and samples were determined in triplicate.

RESULTS & DISCUSSION:

The standard phenol red / chloramine-T method was readily converted from volumetric flasks to microplate scale, producing easy-to-read and easy-to-measure results (Fig. 1). An internal error by Jones (1993) in final reagent concentrations was detected and corrected, resulting in duplication of Jones' reported results (compare Fig. 2 A, B, and C).

To extend the range, a dilution in the microwell was performed by delivering a smaller volume of higher concentration sample and standards. Results remained consistent with the lower concentration range (Fig 2D, upper scale).

The utility of the addition of sodium thiosulfate, part of the original method since 1935, seemed in doubt since Jones' modification. In fact, with the addition of ammonium to the phenol red reagent there appears to be no need to quench the chlorination reaction and paradoxically the addition of sodium thiosulfate appears to destroy the chloramine complex and restart the chlorination of the unreacted phenol red (Fig 3).

Figure 4 shows Br^- breakthrough curves for sand and crushed foundry slag determined using the microplate colorimetric method and ISE.

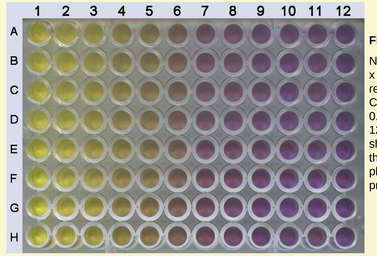


Fig. 1

Ninety-six well microplate (85 x 127 mm) with eight replicates of standards Containing, left to right, 0, 0.5, 1.5, 2.5, 4, 5, 7, 9, 10, 12, 15 and 20 mg L^{-1} Br^-, showing the transition from the yellow color of unreacted phenol red to the reaction product, bromophenol blue.

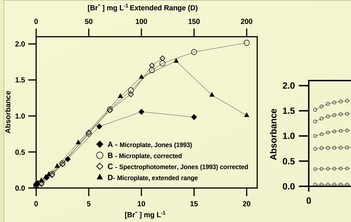


Fig 2 Bromide standard curves: A) Literal recasting of Jones (1993) method to microwells with final reagent concentrations of 0.023 mM PR, 0.148 mM CT); *B*) in microwells with final concentrations of 0.053 mM PR, 0.160 mM CT; *C*) Macroscale version of Jones (1993) with final concentrations as in *B* with absorbance read by spectrophotometer with an optical path of 1-cm; *D*) modification of *B* with a tenfold higher concentration range and 1/10 sample volume.

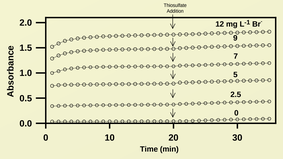


Fig 3 Stability of microplate method colorimetric standards containing 0 to 12 mg L^{-1} Br. Following APHA (1998) and Jones (1993), thiosulfate (TS) is added at 20 minutes (arrows). Upon TS addition, undesirable color development started, even in the 0 mg L^{-1} Br treatment, likely indicating chlorination had resumed. Therefore, the TS addition step was removed from this microplate method.

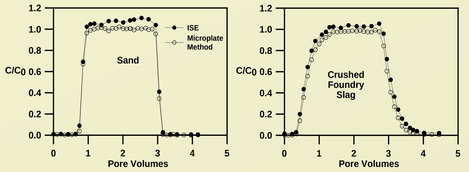
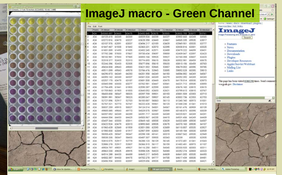
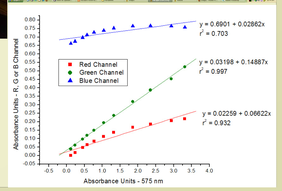


Fig. 4 Bromide breakthrough curves through 75-cm columns filled with sand (left) or crushed foundry slag (right) measured with ISE and the extended range microplate colorimetric method [$C_0 \approx 100$ mg L^{-1} Br^-, [PR] =0.2 mM , [CT] =1 mM]. By the colorimetric microplate method, coefficients of variance for 5 measurements between 1.5 and 2.5 pore volumes were 0.73% and 0.38% for sand and slag, respectively.

SUMMARY AND CONCLUSION:

- Simple and rapid microplate method for colorimetrically determining [Br^-] presented
- Recasting the technique of Jones (1993) to microplates, the range extends to 12 mg L^{-1}.
- With minor modifications, the method can resolve Br concentrations up to 300 mg L^{-1} without a prior sample dilution and volumes as small as 20 µL.
- Method compares favorably with bromide-specific electrodes.
- This method makes determination of Br significantly faster and cheaper, promoting better lab practices with regard to calibration curves and sample replication.





Colorimetry using a desktop scanner in transparency mode and automated image analysis with an ImageJ macro. More info coming in the near future!
(See conference presentation 318-7, 11:15 Wed; presenter D. Soldat)

References:

APHA, AWWA, and WPCF. 1998. Standard methods for the examination of water and wastewater. 20th ed. American Public Health Association, Washington, DC.

Jones, David R. 1993. Improved spectrophotometric method for the determination of low levels of bromide. Anal. Chim. Acta 271:315-323.

Lepore B. J. and P. W. Barak. 200_ . A Colorimetric Microwell Method for Determining Bromide Concentrations. Submitted to Soil Sci. Soc. Am. J.

Stenger, V.A. and I.M. Kolthoff. 1935. J. Am. Chem. Soc. 57:831-833.

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