ELSEVIER

Journal of Chromatography A, 671 (1994) 197–203

JOURNAL OF CHROMATOGRAPHY A

Ion chromatography with UV detection for the determination of thiosulfate and polythionates in saline waters

S.I. Weir*[,a], E.C.V. Butler[b], P.R. Haddad[c]

[a]*Institute of Antarctic and Southern Ocean Studies, University of Tasmania, Hobart, Tasmania, Australia*
[b]*CSIRO Division of Oceanography, Marine Laboratories, GPO Box 1538, Hobart, Tasmania 7001, Australia*
[c]*Department of Chemistry, University of Tasmania, Hobart, Australia*

Abstract

A high-performance liquid chromatographic method was developed for the determination of the sulfur oxy-anions, thiosulfate and polythionates, in natural saline waters. This method utilises preconcentration techniques to effectively enrich the analytes whilst discriminating against the high chloride concentrations present, and is based on the novel combination of an ion-exchange pre-column in succession with a reversed phase analytical column and an eluent based on a water–acetonitrile mixture containing tetrabutylammonium ions and carbonate buffer. The limit of detection is 1 n*M* for trithionate and 0.3 n*M* for tetrathionate and pentathionate when concentrating 6 ml of 1:50 diluted seawater. The method has a precision of 0.25% for concentrations of 1 μM. Analysis time is approximately 30 mins.

1. Introduction

The analysis of mixtures of sulfur oxyanions in aqueous solution is a valuable and necessary tool for investigating the chemistry of sulfur-rich waste water effluents such as mining and milling wastes, oil-shale retort wastes, paper and pulp wastes, and acid-mine drainage. Hydrochemical processes also involve mixtures of dissolved sulfur species in various oxidation states, *e.g.*, redox processes in sulfur-rich ground waters and geothermal waters, pyrite oxidation in alkaline waters and sulfur oxidation in soils [1].

The sulfur oxy-anions thiosulfate ($S_2O_3^{2-}$) and the polythionates ($S_nO_6^{2-}$) are important intermediates in aquatic biogeochemical processes such as the oxidation of elemental sulfur and reduced sulfur species. Thiosulfate is also a common product of the inorganic oxidation of sulfide ions and iron sulfides, as well as the disproportionation of bisulfite and sulfite ions. Both thiosulfate and polythionates are oxidised or reduced by several groups of bacteria [2].

The study and quantitation of these compounds can provide clues to determine the rates and pathways of chemically and biologically mediated sulfur transformations in aquatic systems. A convenient technique for the determination of these compounds is ion chromatography, in particular the use of reversed phase columns with ion-interaction reagents [3–6]. Of the procedures developed, however, none are

* Corresponding author. Present address: CSIRO Division of Oceanography, Marine Laboratories, GPO Box 1538, Hobart, Tasmania 7001, Australia.

0021-9673/94/$07.00
SSDI 0021-9673(93)E1132-J

directly applicable to the analysis of these compounds in saline waters. Interference from the high concentrations of chloride in the sample is the problem. This paper describes a method of analysis for thiosulfate and polythionates in saline waters.

2. Materials and methods

2.1. Instrumentation

The ion chromatographic system was composed of an ETP Kortec K35D HPLC pump, a Rheodyne 7125 injector with a 200-μl sampling loop and a SPD-10AV Shimadzu UV–Vis spectrophotometric detector. Samples for preconcentration were loaded onto the precolumn by way of a Rheodyne 7000 six-port switching valve which was operated manually. The analytical column was a Hamilton PRP-1 reversed-phase column, 10 μm particle size (150 × 4.1 mm) and was protected by a Brownlee PRP-1 guard cartridge (PRP-GU, 30 × 4.6 mm) in a Brownlee 30 mm MPLC cartridge holder. The precolumn used for sample preconcentration was a Waters IC-PAK, 10 μm particle size (5.0 × 6.0 mm) housed in Waters Guard-Pak precolumn module. Chromatograms were displayed on a Yew type 3056 dual-pen recorder; the detector output was also fed, via an analog-to-digital interface, to an IBM-style personal computer for processing with integration and data analysis software (DAPA SCIENTIFIC PTY LTD, Kalamunda, Australia).

2.2. Reagents

The mobile phases used for ion-interaction separations comprised water treated with three-cartridge Milli-Q water purification system, acetonitrile (ACN), Waters low-UV PIC-A ion-interaction reagent, sodium carbonate, sodium hydrogencarbonate and sodium chloride. Eluents were prepared by diluting the acetonitrile (expressed as a percentage of total volume[a]), ion-interaction reagent, buffers and sodium chloride with Milli-Q water to volume. Eluent was filtered through a 0.45-μm membrane filter and degassed by ultra-sonicating under vacuum. Stock solutions of thiosulfate and polythionates were prepared by dissolution of an accurately weighed amount of salt in Milli-Q water. Analytical-grade sodium thiosulfate was obtained from Ajax Chemicals, sodium tetrathionate dihydrate was obtained from Fluka. Potassium trithionate and potassium pentathionate were synthesised [7].

2.3. Chromatographic procedures

General procedure: All chromatographic separations were carried out at room temperature (20 ± 2°C) using an eluent flow-rate of 0.5 ml/min. Dilute standard solutions prepared in eluent were injected directly onto the column. Analytical and concentrator columns were equilibrated with eluent. Equilibration was established when a steady baseline was observed and retention of a standard solution was reproducible. Detector wavelength was set at 205 nm.

2.4. Sample pre-concentration

Pre-concentration of samples was carried out using a six-port switching valve (Fig. 1) with flow paths varied according to the following sequence.

(A) Equilibration of the columns. With both the concentrator column and the analytical column in the eluent flow path (INJECT position), eluent was pumped through the system at 0.5 ml/min.

(B) Loading sample onto concentrator column. The switching valve was rotated to place the concentrator column off line while the analytical column remained in the eluent flow path (LOAD position). The sample volume was then loaded, followed by a wash step.

(C) Elution of sample from concentrator column. The switching valve was rotated to the position used in A above and solutes were eluted

[a] Reference to % modifier as ACN–water (20:80) indicates 200 ml of ACN made up to 1000 ml with Milli-Q water.

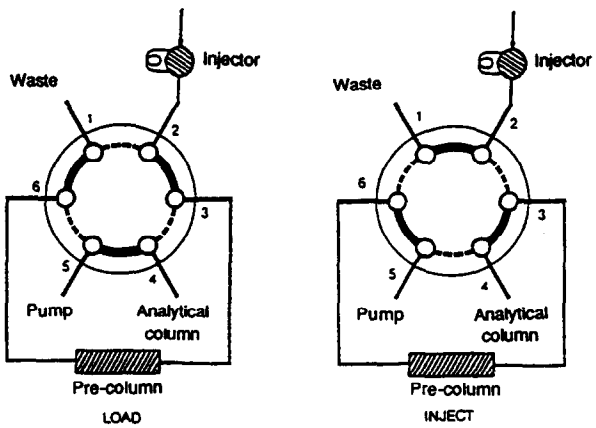

Fig. 1. Switching valve showing the load and inject positions.

from the concentrator column and carried to the analytical column for subsequent separation.

3. Results and discussion

3.1. Initial experiments

The chromatographic conditions selected to achieve the resolution of thiosulfate and polythionates ($n = 3$–5) were based on those used by Steudel and Holdt, [4] and were as follows; acetonitrile–water (25:75), 1.5 m*M* low-UV PIC-A, 0.3 m*M* sodium carbonate and 0.3 m*M* sodium hydrogencarbonate. Before proceeding with the sample preconcentration approach, the existing direct injection ion-interaction method was modified to maximise sensitivity for the separation of thiosulfate and polythionates (Fig. 2).

3.2. Choice of ion-pair reagent

One of the key eluent parameters that governs retention is the length of the carbon chains on the ion-interaction reagent (IIR). In general, as the carbon chain number increases, retention of the R_4N^+ salt increases [8]. Three alkyl chain lengths were tested, tetrapropylammonium (TPrA), tetrabutylammonium (TBA) and tetrapentylammonium (TPeA) all with the phosphate counter anion. Initial experiments using 1.5 m*M* TPeA·PO_4 exhibited a capacity factor at least twice that observed with TBA·PO_4, thus making TPeA·PO_4 undesirable as an ion-pair reagent for the polythionates under the conditions used. Further experiments using TPrA·PO_4

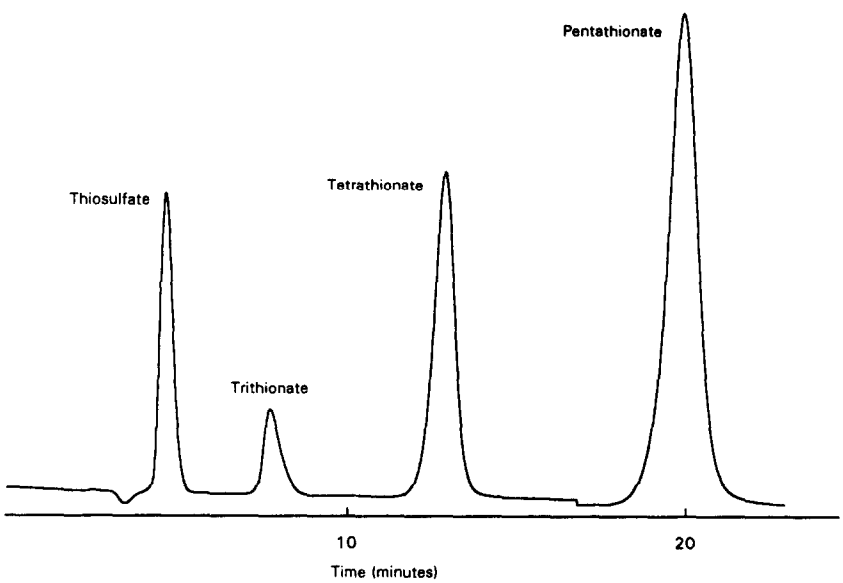

Fig. 2. Injection of 200 μl of a mixed standard of 10 μM of thiosulfate, trithionate, tetrathionate and pentathionate. Column: Hamilton PRP-1, reversed phase 5 μm particles, 150 × 4.1 mm. Eluent: ACN–water (25:75), 1.5 m*M* low-UV PIC-A, 0.6 m*M* carbonates. Detection: 205 nm, 0.04 AUFS.

were also unsuccessful, an eluent of 1.5 m*M* TPrA·PO_4 in ACN–water (28:72) gave little retention, with the early species eluting with the solvent front. Thus the TBA·PO_4 proved to be the most effective IIR for the separation of thiosulfate and the polythionates under the conditions used.

Effects of the counter ion have been described by Iskandarani and Pietrzyk [8]. Comparison of the TBA·OH, TBA·Ac and Waters low-UV PIC-A (incorporating TBA–HSO_4) found all to be suitable for separation with slight alteration of conditions. However, low-UV PIC-A was the most suitable because of the lower background absorption of this reagent at 205 nm.

Although PIC-A was determined to be the most suitable IIR for this study, previous studies using the IIR tetrabutylammonium acetate found it to satisfactorily separate the analyte species in the presence of chloride within a shorter retention time. The hygroscopic nature of this reagent however limited its use for precise eluent preparation.

3.3. On-column matrix elimination

In contrast to the specific methods of matrix elimination whereby matrix ions are reduced or removed prior to analysis using pre-columns or membranes, a more general approach described as on-column elimination [9,10] involves the use of the major matrix ion as the eluent. When this is done the column is effectively in the form of the matrix ion and therefore shows little or no retention of this anion when it is directly injected. Since the purpose of these studies was to find chromatographic conditions which tolerated high levels of chloride, it was necessary to employ a detection method which shows little response to this species. UV absorbance detection was well suited for this purpose.

Optimal eluent conditions were sought for the separation of the thiosulfate and polythionate anions using chloride as the matrix anion. On-column matrix elimination was shown to be effective in reducing the effects of direct injections of up to 0.1 *M* sodium chloride. A plot of log retention time *versus* log sodium chloride concentration included in the eluent, revealed

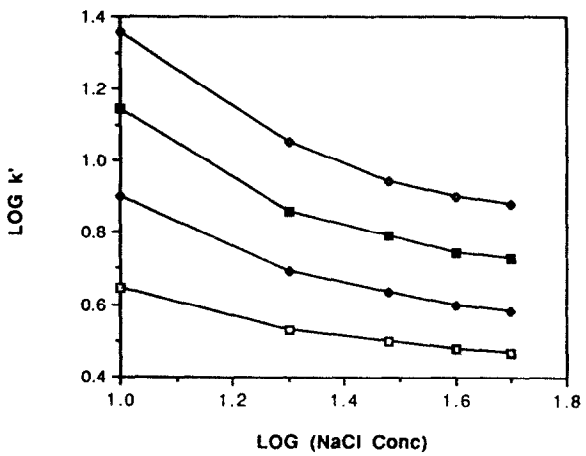

Fig. 3. Variation of solute retention with the concentration of chloride in the eluent. □ = S_2O_3; ◆ = S_3O_6; ■ = S_4O_6; ◇ = S_5O_6.

the optimal eluent to contain sodium chloride in the concentration range of 30–45 m*M* (Fig. 3).

The higher level of chloride included in the eluent (45 m*M*) would be expected to result in satisfactory separation of analytes in saline standard solution. However, a lower value was adopted to ensure solutes did not elute too close to the solvent front. The optimal eluent was therefore determined to be ACN–water (25:75), 1.5 m*M* low-UV PIC-A, 0.6 m*M* carbonates (3 m*M* sodium carbonate, 0.3 m*M* sodium hydrogencarbonate) and 35 m*M* NaCl. The chromatogram obtained with this eluent is shown in Fig. 4.

3.4. Pre-concentration

Eluent composition and concentrator column characteristics were selected to discriminate against the retention of chloride on the pre-concentration column. The eluent is required to remove the analyte species from the pre-concentration column in minimum time, and as quantitatively as possible (*i.e.* 90% removal in 200 μl). In addition, analyte anions should elute late from the analytical column to facilitate ease of preconcentration.

Prior to preconcentration procedures, the eluent composition for optimum separation of analyte anions, thiosulfate and polythionates

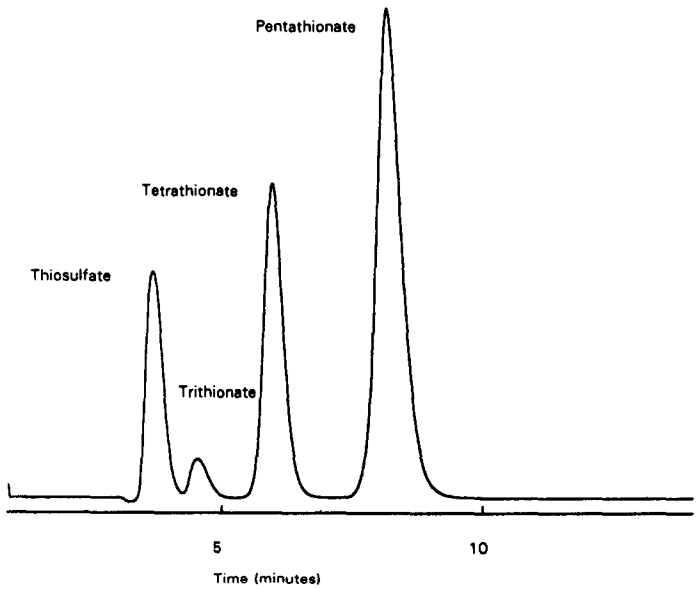

Fig. 4. Separation of a 200-μl injection of 10 μM of thiosulfate and the lower polythionates trithionate, tetrathionate and pentathionate. Column: Hamilton PRP-1, reversed phase 10 μm particles, 150 × 4.1 mm. Eluent: ACN–water (25:75), 1.5 mM low-UV PIC-A, 0.6 mM carbonates and 35 mM sodium chloride. Detection: 205 nm, 0.04 AUFS.

($n = 3$–5), was determined to be ACN–water (25:75), 1.5 mM low-UV PIC-A, 0.6 mM carbonates and 35 mM NaCl. Preliminary experiments showed the polythionate anions to be tightly bound to the ion exchange resin in the concentrator column when in the presence of chloride, with thiosulfate being less well retained. The eluent was therefore required to have a high eluting capacity in order to transfer the solute anions from the concentrator column, yet still retain the properties which enable effective separation and determination of the analyte anions on the analytical column. These eluent requirements were met by the addition of the UV transparent anion, perchlorate (ClO_4^-), the most effective counter ion at reducing retention times [11]. Reoptimisation of the eluent was therefore required and is summarised below:

(1) Addition of ClO_4^- was required to strip analytes from the pre-column; this change decreased the retention time for all species on the analytical column.

(2) In order to counteract the decreased retention times observed after the addition of the perchlorate anion, both the carbonate and the IIR concentrations were increased. This resulted in increased retention times for all species.

(3) The concentration of sodium chloride was decreased marginally to improve separation between trithionate and an early eluting broad peak. This modification also resulted in increased retention times for all species.

The optimised eluent which effectively stripped the analyte from the pre-column prior to successful separation on the analytical column was determined to be: ACN–water (25:75), 2 mM carbonates (1 mM Na_2CO_3, 1 mM $NaHCO_3$), 30 mM NaCl, 10 mM low-UV PIC-A and 3 mM $NaClO_4$. A typical chromatogram obtained for the preconcentration of 6 ml of a 1 μM mixed polythionate standard solution is given in Fig. 5.

Breakthrough experiments were performed in order to determine the length of time the solute ions, thiosulfate and trithionate, were held on the concentrator column, which in turn was influenced by the concentration of chloride ion present. There was no need to test the higher polythionates as they would be more effectively retained on the column than trithionate. Thiosul-

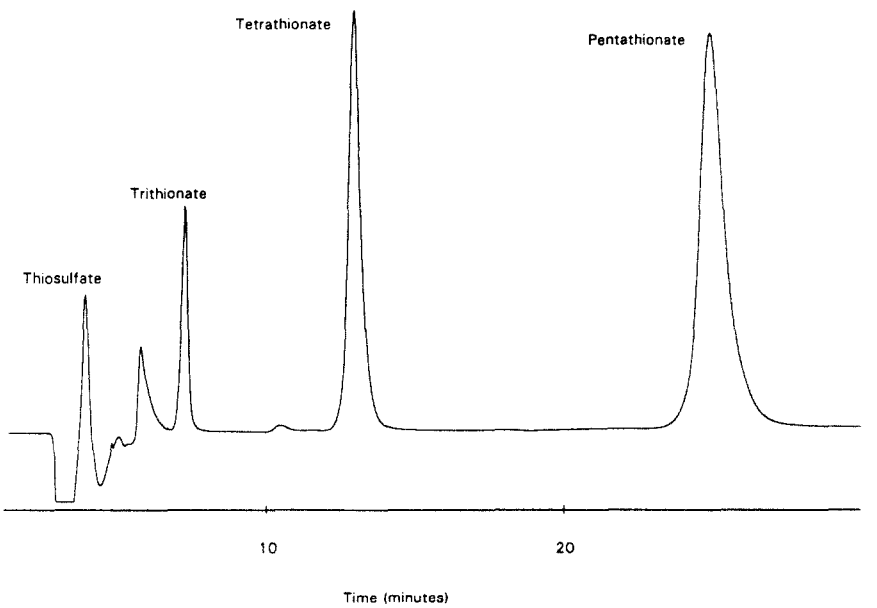

Fig. 5. Separation of a 6-ml sample of 1 μM of thiosulfate and the lower polythionates trithionate, tetrathionate and pentathionate. Column: Hamilton PRP-1, reversed phase 10 μm particles, 150 × 4.1 mm. Eluent: ACN–water (25:75), 10 mM low-UV PIC-A, 3 mM ClO_4^-, 30 mM NaCl, 2 mM carbonates. Detection: 205 nm, 0.05 AUFS.

fate showed significantly smaller breakthrough times than trithionate for the same concentration of sodium chloride. The optimum sodium chloride concentration for preconcentration, at which analytes could still be effectively retained on the concentrator column, was determined to be 0.01 M. Therefore sodium chloride solutions higher than this concentration would be required to be diluted to this level. Approximately 9.0 ml of sample can be injected before 2 mM trithionate breaks through the concentrator column. However, a considerably smaller volume can be injected if thiosulfate is still to be retained by the concentrator column due to the low affinity this species has for the resin packing.

Empirical trials were set up to investigate the following: (a) the volume of sample loaded onto the pre-column and (b) the type and volume of solution used in the wash step during sample loading, with the following results.

(a) The sample volume load employed for trial studies was 6.0 ml. This volume resulted in the successful determination of the polythionates ($n = 3$–5), however, the thiosulfate peak definition was lost in a large system peak, evident in Fig. 5.

(b) Trials utilising the following: no wash step, wash step using diluted sodium chloride solution, and wash step using Milli-Q water, found the latter to provide the best separation. The sodium chloride solutions served to mask the trithionate peak in the separation, while no wash step resulted in a peak approximately half the area of that peak obtained using the Milli-Q wash solution. The volume adopted for trial studies was 3.0 ml.

3.5. Performance characteristics

Precision was assessed by repeatedly injecting samples of the mixed standard solution, with each component at a concentration of 1 μM. For 20-μl injections, the relative standard deviation ($n = 6$) for the polythionates was 0.25%. The detection limit for trithionate was calculated to be 1 nM for a 6-ml sample volume of 0.01 M NaCl mixed standard loaded onto the precolumn (Detection limits were defined as two times baseline noise). Detection limits for tetrathionate and pentathionate were approximately three times more sensitive. While lower detection limits would be achievable with larger injection

volumes, this would be at the expense of thiosulfate which would be lost in the separation. Analytes were pre-concentrated by a factor of 10 (relative to direct injection) when a 6-ml volume sample was loaded.

4. Conclusions

An ion chromatography method was developed for the determination of the sulfur oxyanions, thiosulfate and polythionates, in saline waters. This method utilises preconcentration techniques to effectively enrich the analyte sample whilst discriminating against the high chloride concentrations present, and is based on the novel combination of an ion-exchange pre-column in succession with a reversed-phase analytical column and an eluent based on a water–acetonitrile mixture containing tetrabutylammonium ions and carbonate buffers. Baseline resolution of the polythionates ($n = 3$–5) was achieved in the elution order trithionate, tetrathionate and pentathionate in the presence of 0.01 M sodium chloride.

5. References

[1] C.O. Moses, D.K. Nordstrom and A.L. Mills, *Talanta*, 31 (1984) 331–339.
[2] J.O. Nriagu, R.D. Cocker and A.L.W. Kemp, *Limnol. Oceanogr.*, 24 (1979) 383–389.
[3] S.B. Rabin and D.M. Stanbury, *Anal. Chem.*, 57 (1985) 1130–1132.
[4] R. Steudel and G. Holdt, *J. Chromatogr.*, 361 (1986) 379–384.
[5] B. Takano and K. Watanuki, *Talanta*, 35 (1988) 847–854.
[6] B. Takano, M.A. McKibben and H.L. Barnes, *Anal. Chem.*, 56 (1984) 1594–1600.
[7] F. Feher, *Handbook of Preparative Inorganic Chemistry*, Vol. 1, Academic Press, New York, 2nd ed., 1975, pp. 397–402.
[8] Z. Iskandarani and D.J. Pietrzyk, *Anal. Chem.*, 54 (1988) 2427–2431.
[9] K. Ito and H. Sunahara, *J. Chromatogr.*, 502 (1990) 121.
[10] Marheni, P.R. Haddad and A.R. McTaggart, *J. Chromatogr.*, 546 (1991) 221–228.
[11] P.R. Haddad and P.E. Jackson, *Ion Chromatography: Principles and Applications*, Elsevier, Amsterdam, 1990, p. 177.