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Short Communication

Reversed-phase high-performance liquid chromatographic determination of sulphide in an aqueous matrix using 2-iodo-1-methylpyridinium chloride as a precolumn ultraviolet derivatization reagent

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ABSTRACT

A high-performance liquid chromatographic method was developed for the determination of sulphide in water. The procedure involves precolumn derivatization of sulphide with 2-iodo-1-methylpyridinium chloride to form 1-methyl-2-thiopyridone, followed by reversed-phase HPLC separation and UV detection. A linear calibration graph was obtained over the range 0.04–50 μg of S^{2-} in 10 ml of final analytical solution and the relative standard deviations were 0.2% at the 1 μg/ml and 3.2% at the 10 μg/l sulphide levels. The proposed method is tolerant towards thiols and many common cations and anions.

INTRODUCTION

Sulphide is formed throughout nature and industry. In nature it is often produced by the bacterial action on sulphur compounds present in organic wastes and by reduction of sulphate. The main industrial sources of sulphide are kraft pulp mills, petroleum refineries, the gasification of coal, meat processing plants and sewage treatment plants. This sulphide is readily converted into hydrogen sulphide which causes odour, corrosion and toxicity problems. The toxicity of the sulphide ion is caused by its great ability to coordinate with many metals involved in human metabolism. For these reasons, the determination of sulphide assumes considerable importance and many methods have been developed.

Most of the methods published up to 1976 have been described in several books (*e.g.*, refs. 1 and 2). More recent methods for the determination of sulphide at trace levels are instrumental, including the use of spectrophotometry [3–8], inductively coupled plasma atomic emission spectrometry [9], polarography [10,11], potentiometry with ion-selective electrodes [12–14], cathodic stripping voltametry [15,16], spectrofluorimetry [17–19] and flow-injection analysis

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with various detection procedures [20–24]. Chromatographic methods have also been reported, including gas chromatography [25–27], ion chromatography [28–34] and reversed-phase HPLC [35].

Sulphide is difficult to determine by ion chromatography, as explained by Haddad and Heckenberg [35]. They demonstrated that sulphide exists in solution as neutral hydrogen sulphide if the solution is not alkaline. Even if the mobile phase is a solution of sodium hydroxide, sulphide exists mainly as HS^- and is weakly retained on anion-exchange columns. Detections of sulphide also causes problems. Conductimetric detection excludes suppressed ion chromatography and the sensitivity attainable is relatively poor. Potentiometric detection with a silver sulphide ion-selective electrode [32] and amperometric detection using gold [30] and mercury-coated platinum [31] or silver [29] electrodes are very good methods in terms of low detection limits, but there can be problems with non-linearity of calibration at low concentrations. Further, sulphide can be adsorbed on the ion-exchange columns used and oxidized prior to and during the chromatographic analysis.

This paper describes a reversed-phase HPLC method for the determination of sulphide in an aqueous matrix after its reaction with 2-iodo-1-methylpyridinium chloride to form 1-methyl-2-thiopyridone. This procedure converts sulphide into a stable product and permits UV detection.

EXPERIMENTAL

Chromatographic apparatus

The HPLC apparatus consisted of a Hewlett-Packard Model 1050 isocratic pump, a Hewlett-Packard Model 1050 variable-wavelength detector and a Model 4100 line recorder (Laboratórní Přístroje, Prague, Czech Republic). The samples were injected using a Rheodyne Model 7125 injection valve fitted with a 20-μl loop. The column used was 5-μm ODS-2 (125 × 4.0 mm I.D.), operated at a flow-rate of 0.7 ml/min. UV spectra were recorded on a Carl Zeiss Jena UV–Vis spectrophotometer (1-cm cells).

Chemicals and reagents

Chemicals of analytical-reagent grade were used as received. Other chemicals were purified by distillation or recrystallization.

2-Iodo-1-methylpyridinium chloride. The reagent was prepared by quaternization of 2-chloropyridine with methyl iodide and subsequent halogen exchange. A mixture of 2-chloropyridine (15.0 g, 132 mmol) and methyl iodide (20.0 g, 140 nmol) were heated (oil-bath, 75°C) overnight. A yellow precipitate appeared gradually. The cooled mixture was filtered and washed with diethyl ether. Triple recrystallization from ethanol gave 24.21 g (72%) of white needles, which could be resolved into pure 2-iodo-1-methylpyridinium chloride (m.p. 208–209°C).

For sulphide derivatization prior to HPLC, a 0.01 *M* aqueous solution of 2-iodo-1-methylpyridinium chloride was used.

1-Methyl-2-thiopyridone. The compound was prepared as described previously [5].

Buffer solutions. Borate and phosphate buffers of concentration 0.1 *M* were used throughout.

Standard sulphide solution. A sulphide solution (*ca.* 0.04 *M* in 0.04 *M* sodium hydroxide) was prepared from freshly washed large crystals of $Na_2S \cdot 9H_2O$ after absorption of the water with filter-paper. The solution was standardized by the *o*-hydroxymercuribenzoate method. Working standard solutions of sulphide in the range 0.1–10 μg/ml were prepared just before measurement by appropriate dilution with water.

Mobile phase and sample preparation. The mobile phase was methanol–0.005 *M* aqueous KH_2PO_4 (pH 4.7) (20:80, v/v) at ambient temperature. The solvents and the solutions of samples were filtered through a 0.45-μm membrane filter and degassed before use.

Sample derivatization

In a 10-ml calibrated flask were placed 1 ml of working reagent solution and an aliquot of sample, then 3 ml of buffer solution (pH 8) were added. The flask was stoppered, mixed by inversion and put aside for 30 min. The mixture was then diluted to the mark with water and a 20-μl

aliquot was injected into the liquid chromatographic system. The derivatization procedure was applied to working standard solutions of sulphide to obtain a calibration graph.

Assay procedure

An aliquot of the sample solution was subjected to the derivatization procedure and 20 μl of the final analytical solution were injected into the liquid chromatograph in triplicate. Unknown samples were run concurrently with standard solutions. The peak height was measured and the amount of sulphide in each of the samples was then calculated by interpolation on the calibration graph.

Chromatography

Chromatographic separations were carried out under isocratic conditions on an ODS-2 reversed-phase column. For routine determination of sulphide, a mobile phase consisting of methanol–0.005 *M* KH_2PO_4 (pH 4.7) (20:80, v/v) at a flow-rate of 0.7 ml/min and a detector wavelength of 340 nm were found to be appropriate, allowing an adequate separation of the sulphide adduct from excess of the derivatization reagent and the other possible reaction mixture components, *e.g.*, thiol adducts.

RESULTS AND DISCUSSION

The reaction of 2-iodo-1-methylpyridinium chloride with sulphide was first carried out on a preparative scale and the isolated reaction product, 1-methyl-2-thiopyridone, was found to be in agreement with assigned structure. Details of this experiment have been described in a previous paper [5] devoted to a spectrophotometric method for determining sulphide. The same paper presented details of the reactions on an analytical scale for derivatization purposes.

Experiments were carried out to determine the minimum reaction time required to produce the maximum peak height for a given sulphide concentration, and it was found that for 40 ng of sulphide in a 10-ml reaction vessel the maximum peak height was obtained after 30 min. Therefore, this time was applied in all subsequent determinations.

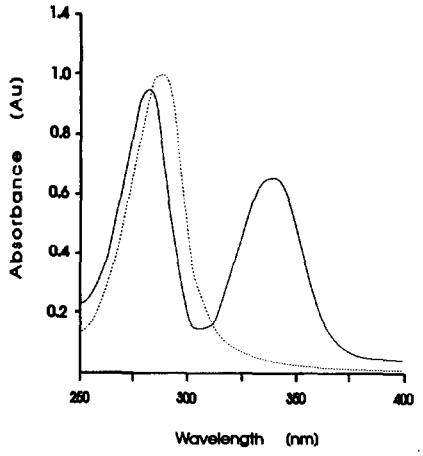

Fig. 1. Absorption spectra of 1.6 μg of sulphide (solid line) and a blank solution (broken line) treated according to the proposed derivatization procedure. Cells with an optical path length of 1 cm were used.

Fig. 1 shows the absorption spectra of a sulphide sample and a blank solution, treated according to the procedure described under Experimental. These spectra show that the reaction product, 1-methyl-2-thiopyridone, had two absorbance maxima at 272 and 340 nm with molar absorptivities of $9.5 \cdot 10^3$ and $7.4 \cdot 10^3$ l mol^{-1} cm^{-1}, respectively. It was therefore possible to monitor the reaction product at both points by selecting the appropriate wavelength according to the sample matrix composition.

Fig. 2 shows a chromatogram obtained for 38 ng of sulphide in final volumes of 10 and 1 ml with detection at 340 nm. 1-Methyl-2-thiopyridone was eluted as a well resolved, slightly tailed peak at a retention time of 4.2 min. Injection of an authentic sample of 1-methyl-2-thiopyridone gave the same peak.

When a detection wavelength of 272 nm was used, the height of the analyte peak increased in accordance with the molar absorptivity (Fig. 1). Excess of reagent was eluted in the form of broad peak at a retention time of about 8 min; at 340 nm the peak could not be seen.

Analytical parameters

A linear calibration graph was obtained over the range 0.04–50 μg of S^{2-} (4 μg/l–5μg/ml)

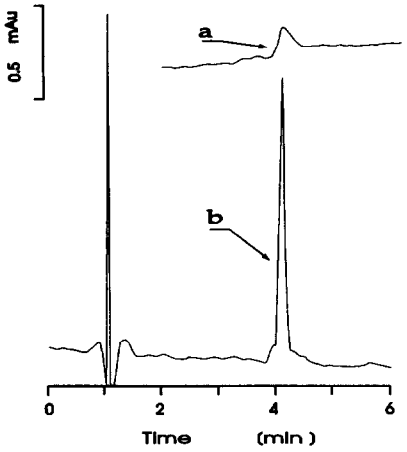

Fig. 2. Chromatogram of 38 ng of sulphide in standard solution derivatized to form 1-methyl-2-thiopyridone, (a) in 10 and (b) in 1 ml of final analytical solution. Conditions: column, 5-μm ODS-2 (125 × 4 mm I.D.); mobile phase, methanol–0.005 *M* KH_2PO_4 (pH 4.7) (20:80, v/v) at a flow-rate of 0.7 ml/min; injection volume, 20 μl; detection, 340 nm.

with relative standard deviations of 0.2% at the 1 μg/ml and 3.2% at the 10 μg/l sulphide level (Table I). The detection limit, determined as the concentration of sulphide in the final analytical solution (using a 20-μl injection) giving a signal equal to three times the baseline noise, was 2 μg/l. The average recovery obtained for a 100 μg/l standard solution of sulphide was 97%. Once formed, 1-methyl-2-thiopyridone, was stable for several weeks in the final analytical solution.

TABLE I

DETERMINATION OF SULPHIDE AS 1-METHYL-2-THIOPYRIDONE

Detection wavelength, 340 nm.

Taken (μg in 10 ml)	Found[a] (μg)	Error (%)	S.D. (μg)	R.S.D. (%)
0.058	0.055	−4.4	0.005	8.6
0.116	0.112	−3.4	0.003	2.8
0.232	0.226	−2.7	0.003	1.3
0.580	0.597	2.96	0.012	2.0
1.16	1.149	−1.0	0.013	1.1
5.8	5.787	−1.3	0.016	0.3
11.6	11.617	1.7	0.015	0.2

[a] Mean result, $n = 5$. Regression equation: $y = 2.79x - 0.006$, where y = peak height (mAu) and x = amount taken (μg/10 ml). Correlation coefficient was 0.9997.

Effect of foreign species

Interference studies were conducted for a wide range of species. Common inorganic anions including chloride, iodide, nitrate, carbonate, sulphite, thiocyanate, acetate and phosphate at a 0.1 *M* concentration ($5 \cdot 10^5$-fold excess over sulphide in the final analytical solution) showed no interference. Thiosulphate at the same concentration as sulphide at the 1 μg/ml level gave a 10% positive interference, probably because of partial desulphurization of the thiosulphate ion with formation of 1-methyl-2-thiopyridone. The exact mechanism of this interference will be elucidated by some additional experiments that are outside the scope of this paper. Thiols (*e.g.*, glutathione) react with the reagent to form ionic thio ethers, which are easily resolved from 1-methyl-2-thiopyridone owing to their much shorter retention times under reversed-phase conditions. Interference by thiols was the chief drawback to the spectrophotometric method described previously [5].

CONCLUSIONS

2-Iodo-1-ethylpyridinium chloride appears to be a useful HPLC derivatization reagent, having good reactivity and selectivity towards sulphide under mild reaction conditions. The compound is cheap, soluble in the water, stable and, as a crystalline substance, very easy to handle. 1-Methyl-2-thiopyridone, the derivatization reaction product, is also crystalline and can be used instead of sulphide for calibration graph preparation. Ultraviolet detection provides a reliable, sensitive and simple method for the determination of sulphide in an aqueous matrix. This means of detection is free from the defects in the amperometric [29,30,31] and potentiometric [32] methods mentioned in the Introduction.

The analytical parameters of this method are similar to those of the reversed-phase HPLC modification of the methylene blue method of Haddad and Heckenberg [35]; the precision (R.S.D.) at the 10 μg/l level were 3.2% (this work) and 3.7% [35], and the corresponding detection limits were 2 and 0.8 μg/l. The methylene blue method is more sensitive, but it has been reported that, in some real situations,

preliminary dilution of samples is required [36]. It is known that the rate of colour formation in the methylene blue method is highly dependent on both the temperature and the total volume. The separation of methylene blue is column dependent and cannot be reproduced on other C_{18} columns reported in the original paper [35].

The present method is tolerant to large excesses of thiols and most common cations and anions; only thiosulphate give a positive interference. If excess of thiosulphate is suspected, sulphide must be separated by one of the reported methods [37,38].

In the methylene blue HPLC method iodide interferes strongly by inhibiting colour formation, whereas in the present method it does not. The high selectivity of the method is the main advantage over the spectrophotometric approach. For all these reasons, the HPLC method based on reaction with 2-iodo-1-methylpyridinium chloride is recommended for determining sulphide in a long series of samples. In environmental analysis the derivatization stage can be performed outside the laboratory in order to convert sulphide into stable product as rapidly as possible.

Further applications of 2-halopyridinium salts as derivatization reagents are currently being explored.

REFERENCES

1 J.H. Karchmer, in A.W. Hanley and F.W. Chech (Editors), *The Analytical Chemistry of Sulphur and Its Compounds*, Part I, Wiley-Interscience, New York, 1970, Ch. 5.
2 W.J. Wiliams, *Handbook of Anion Determination*, Butterworth, London, 1979, Ch. 5.
3 S.R. Bahat, J.H. Eckert, R. Gibson and J.M. Goyer, *Anal. Chim. Acta*, 108 (1979) 293.
4 C.F. Wood and I.L. Marr, *Analyst*, 113 (1988) 1635.
5 E. Bald and W. Ciesielski, *Chem. Anal. (Warsaw)*, 33 (1988) 925.
6 N. Balasubramanian and B.S. Kumar, *Analyst*, 115 (1990) 859.
7 T. Koh, Y. Miura, N. Yamamura and T. Takaki, *Analyst*, 115 (1990) 1133.
8 J. Anwar, N. Ajum and M. Faroogi, *J. Chem. Soc. Pak.*, 12 (1990) 107.
9 K. Lewin, J.N. Walsh and D.L. Miles, *J. Anal. At. Spectrom.*, 2 (1987) 249.
10 G. Luter, A.E. Giblin and R. Varsolona, *Limnol. Oceanogr.*, 30 (1975) 727.
11 L.K. Leung and D.E. Bartek, *Anal. Chim. Acta*, 131 (1981) 167.
12 M. Glaister, G.J. Moody and J.D.R. Thomas, *Analyst*, 110 (1985) 113.
13 H. Clyster, F. Adams and F. Verbeek, *Anal. Chim. Acta*, 83 (1976) 27.
14 Y. Asano and S. Ito, *Denki Kagaku Oyobi Kogyo Butsuri Kagaku*, 58 (1990) 1125; *C.A.*, 114 (1991) 108575c.
15 K. Shimizu and R. Osteryoung, *Anal. Chem.*, 53 (1981) 584.
16 S. Jaya, T.P. Rao and G.P. Rao, *Analyst*, 111 (1986) 717.
17 A. Punta, F.J. Barragan, M. Ternero and A. Guiraum, *Analyst*, 115 (1990) 1499.
18 Y. Arikawa and H. Kawai, *Bunseki Kagaku*, 35 (1986) 720; *C.A.*, 105 (1986) 145216w.
19 S. Bottrell, D. Banks, D. Bird and R. Raiswell, *Environ. Technol.*, 12 (1991) 393.
20 X. Qian, Y. Guo, M. Yamada, E. Kobayashi and S. Suzuki, *Talanta*, 36 (1989) 505.
21 O.F. Kamsan, *Anal. Chim. Acta*, 211 (1988) 299.
22 N. Korsakowa, T. Kukina, T. Sushchevskaya and G. Varshal, *Geokhimiya*, (1991) 45.
23 M. Yagoob, M. Anwar, H. Masood and M. Masoom, *Anal. Lett.*, 24 (1991) 581.
24 J. Lima and L. Rocha, *Int. J. Environ. Anal. Chem.*, 38 (1990) 127.
25 F. Caron and J.R. Cramer, *Anal. Chem.*, 61 (1989) 114.
26 L. Goodwin, D. Fracom, A. Urso and F. Dieken, *Anal. Chem.*, 60 (1988) 216.
27 J. May and T. Francis, *Lab. Pract.*, 40 (1991) 65.
28 R.J. Williams, *Anal. Chem.*, 55 (1983) 851.
29 K. Han and W.F. Koch, *Anal. Chem.*, 59 (1987) 1016.
30 A.M. Bond, I.D. Heritage and G.G. Wallace, *Anal. Chem.*, (1982) 582.
31 W.F. Koch, *J. Res. Natl. Bur. Stand.*, 88 (1983) 157.
32 W.-N. Wang, Y.-J. Chen and M.-T. Wu, *Analyst*, 108 (1984) 281.
33 R.E. Pulson and H.M. Berger, *J. Chromatogr. Sci.*, 25 (1987) 409.
34 O.N. Obrezkow, V.I. Shlyamin, O.A. Spigin and Y.A. Zololov, *Mendeleev Commun.*, (1991) 38.
35 P.R. Haddad and A.L. Heckenberg, *J. Chromatogr.*, 447 (1988) 415.
36 K. Grasshoff and K. Chan, *Anal. Chim. Acta*, 53 (1971) 442.
37 *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, DC, 14th ed., 1976, Section 428.9, p. 499.
38 L. Bark and A. Rixon, *Analyst*, 95 (1970) 786.