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Note

Trace determination of sulfide by reversed-phase ion-interaction chromatography using pre-column derivatization

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Sulfide is present in many environmental samples, such as waste waters, where it is often produced from the bacterial reduction of sulfate. This sulfide is readily converted into hydrogen sulfide which causes odour and toxicity problems. For these reasons, the determination of sulfide assumes considerable importance.

Iodine reacts with sulfide in acidic solution and this reaction forms the basis of a titrimetric procedure for sulfide at levels above 1 ppm and in the absence of interfering substances[1]. A more sensitive determination involves reaction of N,N-dimethyl-*p*-phenylenediamine and hydrogen sulfide in the presence of a mild oxidizing agent to produce methylene blue, with spectrophotometric measurement of the amount of methylene blue formed[2]. This reaction, using ferric ion as the oxidant, is shown in Fig. 1; other substituted analogues of *p*-phenylenediamine show a similar reaction. The chief drawback of the spectrophotometric approach is interference by strongly reducing substances which prevent formation of the blue colour, or interference by other ions which also form a blue colour under the reaction conditions used.

Sulfide is difficult to determine by ion chromatography for a number of reasons. First, sulfide exists in solution as neutral hydrogen sulfide (pK_{a1} = 6.99) unless the solution is made alkaline, so anion-exchange separation can be applied successfully only with alkaline mobile phases such as sodium hydroxide. Even under

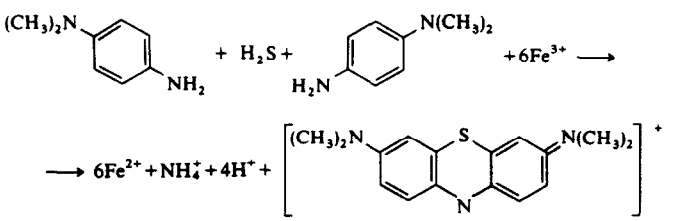

Fig. 1. Reaction of sulfide with N,N-dimethyl-*p*-phenylenediamine and ferric ion to form methylene blue.

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these conditions, sulfide exists predominantly as HS^- and is only weakly retained on anion-exchange columns. Second, sulfide is poorly detected by conductivity measurement when a post-column suppressor device is used because it is converted to a weakly conducting protonated species after passage through the suppressor. Conductometric detection is therefore feasible only for non-suppressed ion chromatography, but the sensitivity attainable by this approach is relatively poor. Finally, sulfide is unstable in aqueous solution and undergoes reaction with oxygen. This reaction can occur both prior to and during the chromatographic analysis.

In view of the limited applicability of conductometric detection, amperometric or potentiometric detection methods are generally recommended for sulfide. Potentiometry using a silver sulfide ion-selective electrode has been reported as a viable detection method for sulfide[3], and amperometric methods using gold[4], mercury-coated platinum[5] or silver[6] working electrodes have also been described. The latter methods exhibit excellent sensitivity for sulfide, with detection limits falling within the 0.1–30 ppb* range, but problems are encountered with non-linearity of calibration at low concentrations and errors due to oxidation of sulfide, impurities in the eluent and adsorption of sulfide onto the ion-exchange columns used.

In this paper we describe a reversed-phase ion-interaction chromatographic method for the determination of trace levels of sulfide after its reaction with N,N-dimethyl-*p*-phenylenediamine to form methylene blue. This process converts sulfide to a stable product and thereby obviates the major problems of the ion-exchange methods. Moreover, the developed method is rapid and simple, and has sensitivity equivalent to that of amperometric detection.

EXPERIMENTAL

Instrumentation and reagents

The liquid chromatograph consisted of a Millipore Waters M590 pump, U6K injector, Model 481 UV–VIS detector operated at 664 nm and M740 data module. The column used was a Waters μBondapak C_{18} (150 × 3.9 mm I.D.), operated at a flow-rate of 1.0 ml/min.

N,N-Dimethyl-*p*-phenylenediamine was obtained from Aldrich (Milwaukee, WI, U.S.A.) and a stock solution was prepared by dissolving 4 g of the free base in 50 ml of 1:1 sulfuric acid. A working solution of the amine was then prepared by diluting 5 ml of stock solution to 100 ml with 1:1 sulfuric acid. Both the stock and working solutions were stored in dark bottles in the refrigerator. The Fe(III) oxidizing solution was prepared by dissolving 25 g of ferric nitrate nonahydrate in 10 ml of water.

A stock solution of sulfide (1000 ppm) was prepared by dissolving an appropriate amount of analytical-grade sodium sulfide nonahydrate in 0.05 *M* sodium hydroxide prepared from boiled-out de-ionized water. This stock solution was stored in a polyethylene bottle in the refrigerator and was used for the daily preparation of standard solutions. These standard solutions were prepared by serial dilution of the

* Throughout the article the American billion (10^9) is meant.

stock solution with de-ionized water (from a Millipore Milli Q apparatus) which had been boiled for 30 min to remove oxygen.

The eluent was prepared by adding 400 ml of HPLC-grade acetonitrile to a 1-litre volumetric flask containing 300 ml of water, after which 5 ml of glacial acetic acid and a vial of Waters low-UV PIC B5 (pentanesulfonic acid) were then added and the flask made up to the mark with water. The eluent was filtered through a 0.45-μm membrane filter and degassed under vacuum in an ultrasonic bath before use.

Procedure

An aliquot (usually 7.0 ml) of sample was added to a 10-ml volumetric flask and 0.5 ml of the working amine solution added with a dispensing pipette, then 3 drops of Fe(III) solution were added with a dropper. The flask was then stoppered and carefully inverted once only. Excessive mixing at this stage can result in loss of hydrogen sulfide, giving low results. The flask was then diluted to the mark with water and allowed to stand for 1 min to permit colour development. After this time, a 100-μl aliquot was injected onto the liquid chromatograph.

RESULTS AND DISCUSSION

Selection of chromatographic conditions

Fig. 2 shows absorption spectra of a 1-ppm sulfide sample and a blank solution, treated according to the procedure described in the Experimental section. These spectra show that the reaction product exhibited maximum absorbance at 664 nm, whilst the unreacted amine showed weak absorbance at 500 nm. It was therefore possible to monitor both the reaction product and the unreacted amine by appropriate wavelength selection.

Methylene blue is a basic compound and under the strongly acidic conditions used in the reaction scheme shown in Fig. 1, it exists as a protonated species. Therefore, an ion-interaction approach using pentanesulfonic acid as the ion-interaction

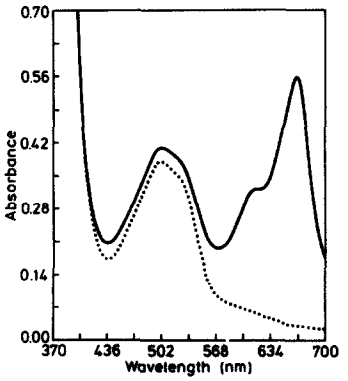

Fig. 2. Absorption spectra of a 1-ppm sulfide standard (solid line) and a blank solution (broken line) treated according to the proposed derivatization procedure. Cells with an optical path length of 1 cm were used.

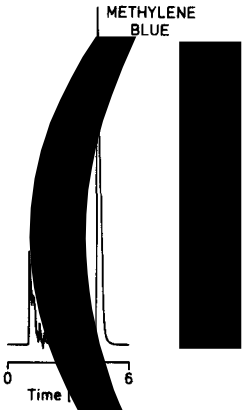

Fig. 3. C atogram of a 1-ppm sulfide standard solution derivatized to form methylene blue. Conditions: column, Waters μBondapak C_{18} (150 × 3.9 mm I.D.); mobile phase, acetonitrile–water (40:60, v/v) containing 0.5% glacial acetic acid and 5 m*M* low UV PIC B5; flow-rate, 1.0 ml/min; injection volume, 100 μl; detection at 664 nm; integrator attenuation, 512.

reagent was investigated for separation of methylene blue from the reaction mixture. Acetic acid was added to the mobile phase in order to ensure the protonation of methylene blue and so increase its retention. Fig. 3 shows a chromatogram obtained for a 1-ppm sulfide solution, using a mobile phase comprising acetonitrile–water (40:60), 0.005 *M* pentanesulfonic acid and 0.5% acetic acid, with detection at 664 nm. Methylene blue was eluted as a well-resolved but slightly tailed peak at a retention time of 4.6 min. Injection of an authentic methylene blue standard solution gave a peak at the same retention time as the major peak in Fig. 3. When the chromatogram illustrated in Fig. 3 was run using a detection wavelength of 500 nm, the height of the methylene blue peak decreased in accordance with the absorption spectra shown in Fig. 2, but the height of the peak at 2.7 min increased dramatically. This result suggested that the peak at 2.7 min was due to unreacted amine.

Studies were undertaken to determine the minimum reaction time required to produce the maximum peak height for a given sulfide concentration and it was found that, for a 1-ppm sulfide solution, the maximum peak height was attained after the solution was permitted to stand for 1 min after dilution to the mark. This time was therefore adopted for all future determinations. The effect of temperature was not examined.

It should be noted that the separation shown in Fig. 3 was column-dependent and could not be reproduced on other C_{18} columns tested. In all cases, other columns gave a very tailed peak for methylene blue and care should therefore be exercised if the developed method is to be applied using alternative columns.

Analytical parameters and interferences

Calibration was found to be linear over the range 1–2000 ppb sulfide and the precision for eight replicate determinations at the 10-ppb level was 3.7% relative standard deviation (R.S.D.). The detection limit, determined as the concentration of sulfide in a 100-μl injection giving a signal equal to three times the baseline noise,

TABLE I

INTERFERENCE EFFECTS FOR SOME INORGANIC ANIONS

Interfering ions were added at the indicated concentration to a 1-ppm sulfide standard and peak areas are expressed as a percentage of the area obtained for a 1-ppm sulfide standard injected alone.

Ion	*Area of methylene blue peak (%)*		
	1 ppm	*10 ppm*	*100 ppm*
$Fe(CN)_6^{4-}$	99.6	95.5	32.1
$Fe(CN)_6^{3-}$	100.2	100.1	118.9
I^-	99.4	33.3	16.1
SCN^-	99.7	99.9	99.2
SO_3^{2-}	99.9	100.3	94.0
$S_2O_3^{2-}$	100.1	100.0	53.1

was 0.8 ppb. The accuracy of the method was 98.7%, as indicated by the average recovery obtained for a 500-ppb standard solution of sulfide. Once formed, the methylene blue was quite stable, with the peak area for a 1-ppm sulfide standard solution remaining constant for at least two weeks after performing the derivatization reaction.

Interference studies were conducted for a wide range of species. Common inorganic anions including chloride, bromide, nitrite, nitrate, phosphate, sulfate, carbonate and cyanide showed no interference at 10 000 fold excess over sulfide. Thiosulfate, thiocyanate, sulfite, iodide, ferrocyanide and ferricyanide are known to interfere with the spectrophotometric method, and quantitative data describing their interference effects on the proposed chromatographic method are given in Table I. At a 10 fold excess over sulfide, only iodide showed appreciable interference by inhibiting colour formation, however at the 100 fold excess level, thiosulfate, iodide and ferrocyanide showed negative interference, whereas ferricyanide gave positive interference.

It is interesting to compare the magnitudes of these interferences with those observed in the spectrophotometric method, for which all of the above ions are considered to be strong interferences, either by inhibition of the formation of methylene blue or by formation of interfering coloured products. Whilst the chromatographic method does suffer interference when formation of methylene blue is inhibited, it is less prone to interferences from other coloured products because these are resolved from methylene blue. For example, ferrocyanide and ferricyanide at the 10-ppm level give intensely blue-coloured solutions and represent major interferences to the spectrophotometric method, but at this level did not interfere with the chromatographic method. A further advantage of the chromatographic method is that the purity of the N,N-dimethyl-*p*-phenylenediamine used was not critical, whereas the opposite is true for the spectrophotometric method in which high blank levels can result from impure amine reagent. In addition, residual iron(III) must be decolourized with phosphate in the spectrophotometric method and this step is unnecessary in the chromatographic approach.

REFERENCES

1 J. P. Danehy and C. W. Zubritsky, *Anal. Chem.*, 46 (1974) 391.
2 *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, DC, 16th ed., 1985, p. 475.
3 W-N. Wang, Y-J. Chen and M-T. Wu, *Analyst (London)*, 109 (1984) 281.
4 A. M. Bond, I.D. Heritage and G. G. Wallace, *Anal. Chem.*, 54 (1982) 582.
5 W. F. Koch, *J. Res. Natl. Bur. Stand. (U.S.)*, 88 (1983) 157.
6 K. Han and W. F. Koch, *Anal. Chem.*, 59 (1987) 1016.