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Journal of Chromatography A, 760 (1997) 319–325

JOURNAL OF CHROMATOGRAPHY A

Short communication

Gas chromatographic determination of nitrite in water by pre-column formation of 2-phenylphenol with flame ionization detection

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Received 4 April 1996; revised 23 July 1996; accepted 23 September 1996

Abstract

Nitrite can be determined by its reaction with 2-aminobiphenyl in acidic medium to produce 2-phenylphenol which is quantified by gas chromatography with flame ionisation detection using biphenyl as an internal standard. The hydrolysis of the intermediate diazonium ion avoids many of the problems encountered in the conventional determination of nitrite by the diazotization of an aromatic amine (usually sulphanilamide) and coupling with N-(1-naphthyl)ethylenediamine dihydrochloride to yield an azo dye followed by spectrophotometry. Unlike this method, the proposed reaction is rapid and does not suffer from interferences by copper(II), iron(III) and lead(II). The calibration graph was linear over the range 5–1000 μg/l NO_2-N and the limit of detection found to be 0.5 μg/l NO_2-N. A single analysis can be completed within 20 min. The method was not affected by coloured or turbid analyte solutions and has been used to determine nitrite in natural waters.

Keywords: Water analysis; Derivatization, GC; Nitrite; Phenylphenol; Inorganic anions

1. Introduction

Nitrite ions cause several noxious effects such as methemoglobinemia, a decrease in oxidative phosphorylation, inhibition of microsomal enzymes, decreases in the efficiency of nutritive diet, etc., and the reaction of nitrite with various amines, endogenous enzymes and amino acids results in carcinogenic, teratogenic and mutagenic N-nitrosoamines [1,2]. The presence of nitrite ions in environmental samples gives an indication of the extent of pollution and eutrophication. The maximum permissible limit in drinking water specified by the European Union (EU) is 100 μg/l nitrite and by the US Public Health Service 60 μg/l nitrite (18 μg/l NO_2-N) [3]. However, the increasing use of nitrite as a food additive for preservation, as in colour fixers, and as a corrosion inhibitor in industrial process water results in levels often being exceeded.

Ion chromatography either alone or coupled with an on-line preconcentration procedure with conductivity detection, direct UV detection, or amperometric detection, is normally the method of choice for the determination of nitrite, and each approach offers acceptable detection limits [4]. The various other methods available for nitrite determination have been reviewed [5,6]. Most of these are batch [7–9], flow injection [10–13] and sequential injection [14] methods based on spectrophotometric detection of intensely coloured azo dyes resulting from the Griess

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PII S0021-9673(96)00801-1

reaction which is carried out in two steps of diazotization of an aromatic amine by the nitrite in acidic medium, and then coupling of the diazonium ion with a phenol or an aromatic amine. Many of these methods have a high sensitivity but require close control of pH and temperature, have relatively long coupling time, and suffer from the interference of copper(II), iron(III), lead(II), sulphide and sulphite. The metal ions can sometimes be masked with EDTA but coloured and turbid solutions are troublesome to analyze as they interfere with the spectrophotometric determination. As an alternative, measuring the decrease in the natural absorbance of 4-nitroaniline on reaction with nitrite avoided the need for a subsequent coupling reaction [15].

An inherent high sensitivity has been found in fluorimetric determinations [6,16,17]. However, one of the main disadvantages associated with most batch fluorimetric methods is the presence of native fluorescing compounds, especially polycyclic aromatic hydrocarbons in water [18,19]. This interference was avoided by reaction of nitrite with 4-methyl-7-aminocoumarin and HPLC of the product to separate from other fluorescing substances [20,21]. Reaction with iodide in the presence of acetone to form iodoacetone followed by GC [22] has also been used for nitrite.

This paper describes a new method for the determination of low μg/l levels of nitrite based on the pre-column diazotization of 2-aminobiphenyl (2-phenylaniline) in acidic medium, and the thermal hydrolysis of the diazo compound to form 2-phenylphenol which is extracted into hexane and analyzed by GC–flame ionization dectection (FID). The effects of interferents are studied and the method is applied to typical natural water samples.

2. Experimental

2.1. Materials

All chemicals used were of >99% purity. The reagent solution (0.03 *M*) was prepared by stirring 2-aminobiphenyl (0.5 g) (Fluka, Buchs, Switzerland) with sulphuric acid–water (1:1 v/v; 10 ml) to effect salt formation and diluting to 100 ml with water. This solution was filtered through a 0.45-μm membrane filter. The internal standard solution was prepared by accurately weighing 0.1 g of biphenyl (Fluka), dissolving in 50 ml of methanol and diluting to 100 ml with water. This solution was diluted with methanol–water (1:1, v/v) to give less concentrated standards.

Sodium nitrite (Fisons, Loughborough, UK) was heated in an air-oven at 105–110°C for 4 h, cooled to room-temperature in a desiccator, and an accurately weighed portion (0.4928 g) was dissolved in 100 ml of water (containing about 10 mg of sodium hydroxide to prevent the formation of nitrous acid, and about 0.1 ml of chromatographic grade chloroform to inhibit bacterial growth) in a standard flask to give a stock solution containing 1000 μg/ml NO_2-N. This solution was sequentially diluted to give less concentrated standards. The stock solution of nitrite was discarded after two days and all diluted solutions were used within 1 h. Solid-phase extraction cartridges (2.8 ml) containing 500 mg of C_{18} sorbent were obtained from Alltech (Deerfield, IL, USA).

2.2. Instrumentation

GC–FID was performed on a 437A Packard gas chromatograph (Chrompack Packard, Delft, Netherlands) equipped with a split injector (ratio 1:30) and an Hewlett-Packard HP 3394A integrator (Palo Alto, CA, USA). The injector, detector and oven temperatures were 210, 240 and 180°C, respectively. Nitrogen was used as a carrier gas with a flow-rate of 6 ml/min. Aliquots (0.5 μl) of the derivatized analyte extract in hexane were injected onto a Econo-Cap SE-54 column (30 m×0.32 mm I.D. with a film thickness of 0.25 μm) (Alltech).

2.3. Analytical procedure

A known volume (0.5–3 ml) of a sample solution containing 0.01–5 μg/l NO_2-N was mixed with 500 μl of 2-aminobiphenyl reagent solution in a 5-ml standard flask. The reaction mixture was kept at room temperature for about 3 min and was then placed in a boiling-water bath for about 5 min. After cooling to room temperature, 200 μl of the biphenyl internal standard solution was added and the contents diluted to the mark with water. Hexane (200 μl) was

added, the flask shaken for about 4 min, allowed to stand for the phases to separate and then a 0.5-μl aliquot of the upper hexane layer was injected into the gas chromatograph.

The interfering organic compounds in the sample were removed by solid-phase extraction before derivatization. A C_{18} cartridge was activated by washing in succession with 2 ml each of methanol and water, and the sample solution (0.5–3 ml) gently passed through it. The sorbent was washed with 500 μl of water and the combined eluate and washings were treated as above.

3. Results and discussion

3.1. The chemistry

Because of the precautions needed to carry out the Griess reaction to determine nitrite the present study examined alternative reaction pathways that would be less susceptible to reaction conditions or interferences.

It was proposed to investigate the reaction of an aromatic amine reagent with nitrite in acidic medium using conditions which would cause hydrolysis of the diazonium ion to yield a phenol instead of its conversion to an azo dye.

3.2. Selection of the derivatizing agent

The initial criteria for selecting the amine reagent were that it should undergo negligible self-coupling during the diazotization reaction and the phenolic product should be readily extractable to enable derivative enrichment. By selecting amines which were sterically crowded around the amino group, the reaction (and self reaction) of the corresponding diazonium compound with an amine or a phenol should be limited. However, the steric effect would not be expected to hinder hydrolysis at elevated temperatures to give the corresponding phenol.

Many phenols are highly polar and have low partition ratios from an aqueous solution into a non-polar solvent – such as hexane which is suitable for GC – and have small breakthrough volumes on solid-phase extraction sorbents. The relative responses of a number of potentially useful phenols following their extraction into hexane from aqueous solution were therefore examined as a guide to their extractability and FID sensitivity (Table 1). 2-Phenylphenol, which would be formed by the reaction of 2-aminobiphenyl, appeared to be a good choice as it had a high relative response compared to most of the other phenols (except for 2-*tert.*-butyl-4-methylphenol), suggesting an efficient extraction, and was well retained by the column.

3.3. Validation of derivatization

Using 2-aminobiphenyl as the reagent for nitrite ions, it was determined that a 3-min period for the diazotization, followed by a hydrolysis period of 5 min in a boiling-water bath, and 4 min shaking with the extraction solvent yielded the optimum response for the 2-phenylphenol in the chromatographic determination. The chromatographic peak for 2-phenylphenol (Fig. 1) was identified by comparison

Table 1
Selection of derivatizing agent on the basis of the phenol formed

Phenol	Retention time (min)	Relative FID[a] response
4-*tert.*-Butylphenol	2.02	0.369
2,6-Dimethylphenol	1.70	0.476
2,3,6-Trichlorophenol	2.41	0.684
4-Chloro-2,6-dimethylphenol	2.13	0.720
2,4,6-Trimethylphenol	1.85	0.785
4-Bromo-2,6-dimethylphenol	2.48	0.826
2-*tert.*-Butyl-4-methylphenol	2.17	2.585
2-Phenylphenol	3.04	1.000

The relative FID responses are with respect to 2-phenylphenol on molar basis.

[a] An aqueous solution (5 ml) containing 5 μg/ml of each of the phenols being tested and 2-phenylphenol was extracted into 200 μl of hexane and a 0.5-μl aliquot of hexane extract injected into the GC system.

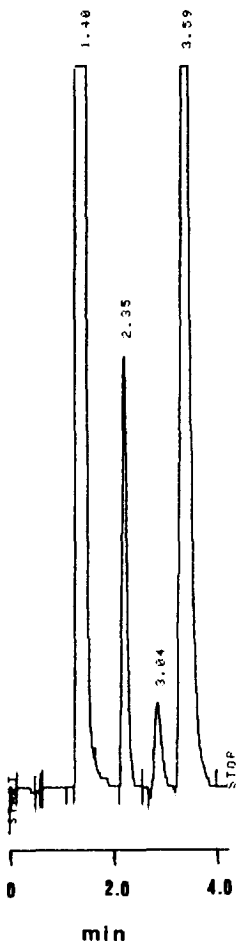

Fig. 1. GC–FID chromatogram of pre-column derivatized 20 μg/l NO_2-N. Peak assignments: 2.35 min=biphenyl (80 μg/l, internal standard); 3.04 min=2-phenylphenol and 3.59 min=2-amino-biphenyl. See Section 2.3 for conditions.

of its retention time with that of the authentic sample. Biphenyl was chosen as the internal standard as it was adequately separated from the analyte and did not interfere with the reaction. Over a range of concentrations of 50, 400 and 800 μg/l NO_2-N, the overall ratio of peak areas derived of equal molar masses of nitrite (after derivatization) and of authentic 2-phenylphenol (relative to the internal standard) was 0.985 (n=9, R.S.D.=4.6%) confirming the quantitative nature of the derivatization procedure.

3.4. Calibration graph and limit of detection

A rectilinear calibration graph was obtained between the amount of nitrite and the ratio of peak areas of the derived 2-phenylphenol compared to the internal standard biphenyl over the concentration range 5–1000 μg/l NO_2-N (Table 2). The limit of detection, calculated as the concentration that gave a signal 3 times the standard deviation of the blank, was found to be 0.5 μg/l NO_2-N. This range and sensitivity compare well with alternative published methods for nitrite (Table 3), many of which require more complex chemical stages or are susceptible to interferences [7,9,17,22–24].

3.5. Validation of the analytical procedure

The proposed method and standard Griess method [25] were applied to the determination of nitrite spiked into deionized water at concentrations of 10, 25, 50, 100, 250, 500 and 750 μg/l NO_2-N. The average recovery in the present method was 97.8% (2.5% R.S.D.) with respect to the results obtained by the Griess method suggesting a comparable efficiency for these aqueous solutions.

3.6. Interferences

3.6.1. Inorganic substances

The interference of a number of ions with the proposed method has been studied by spiking 100 μg/l NO_2-N with known amounts of foreign ions which are likely to occur with nitrite in real samples and which have been reported to interfere with published methods. No attempt was made to pre-separate or mask the interfering ions. The results from the present method showed a different nature and level of interferences compared to methods based on the full Griess reaction. Since the present method only involves a half-Griess reaction, any interactions in this study would have to be occurring with the diazonium cation rather than an azo dye product. As the striking feature of the present method is the absence of interferences from rela-

Table 2
Calibration data for the determination of nitrite (10 calibration points in each range)

μg/l NO_2-N	Slope (%R.S.D.)	Intercept (%R.S.D.)	r
5–100	0.1001 (4.5)	8.5253 (6.2)	0.9988
100–1000	0.1100 (3.1)	1.0166 (5.0)	0.9993

Table 3
Comparison of the present method with other methods of nitrite determination (methods selected from a range of reports giving the best limit of detection for each assay technique)

Technique	Limit of detection[a]	Linear dynamic range[a]	Reference
Present study	0.5	5–1000	
Voltammetry	0.28	1.4–1400	[27]
Potentiometry	70	$0.9–1.4\cdot10^6$	[28]
Fluorimetry	0.3	3–60	[17]
Chemiluminescence	0.1	3–1000	[29]
Capillary electrophoresis	0.3	2.4–23.7	[31]
Spectrophotometry	0.2	0–30	[32]
Ion chromatography	0.06	14–1400	[30]
HPLC	0.15	0.3–9	[20]
GC	2.4	2.4–760	[22]

[a] All concentrations are in μg/l NO_2-N.

tively large concentrations of metal ions (Table 4), this might suggest that their interaction in the full Griess method is due to spectral shift of metal ion–azo dye chelates. In most azo dye methods, sulphite and sulphide ions produce intractable interferences unless they are masked by auxiliary reagents [23,24]; the same problem appeared to occur in the present study (Table 4). It appears that these ions interact directly with the diazonium cation probably through a nucleophilic reaction to give $R–N=N–S^-$ or $R–N=N–SO_3^-$ by analogy with the formation of $R–N=N–O^-$ and R–N=N–NH–R by the reaction of diazonium cations with hydroxide ion and amines, respectively [26]. Presumably, these sulphide and sulphonate ions interfer with the subsequent step of hydrolysis or coupling, or decompose to a thiophenol and arylsulphonate, respectively.

The other major interfering ion was iodide (Table 4), which can react with nitrite to liberate iodine in acidic medium and also form iodo compounds with

Table 4
Study of interferences at 100 μg/l NO_2-N level (the results are the average of 3 determinations)

Interference	Amount spiked, mg/l	% Difference in recovery[a]
Iron(III)	100	1.0
	200	1.5
Copper(II)	10	1.0
	200	1.8
Lead(II)	100	1.6
Calcium(II)	5000	1.4
Aluminium(III)	500	1.1
Mercury(II)	100	1.3
Zinc(II)	500	1.0
Chloride	300	0.8
	1000	−8.5
Bromide	100	1.1
	500	−5.4
Iodide	2	−5.6
Nitrate	1000	0.9
Sulphate	1000	1.3
Phosphate	300	1.8
Sulphite	20	−1.5
	50	−10.2
Sulphide	2	−1.0
	5	−1.2
	10	−11.7

[a] The % difference in recovery is calculated from the amount of nitrite found in the presence and absence of interfering materials.

aryl diazonium ion at elevated temperatures by the Griess replacement reaction [26]. Lowered yields obtained in the presence of large amounts of bromide and chloride can also be traced due to the formation of corresponding halo compounds [26].

3.6.2. Organic substances

Formaldehyde, acetaldehyde, acrylonitrile, ethylene glycol and acetone could be tolerated at high concentrations (100 mg/l) in the determination of 100 μg/l NO_2-N. However, many other organic compounds such as phenols react with nitrite in acidic medium to give nitroso-derivatives or with the diazonium ion to form azo dyes. There were always additional peaks in the chromatogram if the sample was contaminated with unknown organic compounds. A sample clean-up by solid-phase extraction on C_{18} sorbent was therefore usually used to remove the organic interferences. Using this method, the following organic compounds did not produce more than a 2% error when present on 100 μg/l NO_2-N solutions: phenol (10 mg/l), 2,4-dichlorophenol (10 mg/l), picric acid (50 mg/l), nitrobenzene (200 mg/l), benzaldehyde (20 mg/l) and azobenzene (20 mg/l). Solid-phase extraction was also found to be an efficient technique for the removal of colouring matter and may find application in conjunction with batch methods of spectrophotometry.

3.7. Application to the spiked and natural water samples

The present method was applied to the determination of nitrite in spiked and unspiked tap water and pond water samples (Table 5). The overall recovery of added nitrite was found to be 100% (range 96–104%) with 3.2% R.S.D. (range 2.0–4.2%).

4. Conclusion

The proposed method for the determination of nitrite based on the diazotization/hydrolysis of the sterically hindered 2-aminobiphenyl avoids many of the problems encountered with the Griess reaction to form an azo dye. It gave comparable sensitivity and a linear response, and showed only limited problems with interferences, principally with sulphite and sulphide and to a limited extent with halides.

Acknowledgments

Thanks are due to the Commission of the European Communities, Brussels, and the Department of Science and Technology, New Delhi, for award of a Marie Curie Postdoctoral Fellowship to A.J., and to Rani Durgavati University, Jabalpur, India, for leave of absence to K.K.V.

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Table 5
Determination of nitrite in water samples

Sample	Amount of NO_2-N (μg/l)			% Recovery	%R.S.D.
	Added	Found[a]	Recovered		
Tap water	0.0	NF[b]			
	10.0	10.2	10.2	102	2.0
	15.0	14.7	14.7	98	3.3
Pond water	0.0	5.8	4.2		
	10.0	15.5	9.7	97	4.1
	15.0	21.4	15.6	104	3.1

[a] The results are average of 6 determinations.
[b] NF=Not found.

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