A Simple and Sensitive Method for the Determination of Hydroxylamine in Fresh-Water Samples Using Hypochlorite Followed by Gas Chromatography

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A new and simple method for the determination of hydroxylamine in environmental water, such as fresh rivers and lakes using hypochlorite, followed by its gas chromatographic detection, has been developed. A glass vial filled with sample water was sealed by a butyl-rubber stopper and aluminum cap without head-space, and then sodium hypochlorite solution was injected into the vial through a syringe to convert hydroxylamine to nitrous oxide. The head-space in the glass vial was prepared with 99.9% grade N₂ using a gas-tight syringe. After the glass vial was shaken for a few minutes, nitrous oxide in the gas-phase was measured by a gas chromatograph with an electron-capture detector. The dissolved nitrous oxide in the liquid-phase was calculated according to the solubility formula. The proposed method was applied to the analysis of fresh-water samples taken from Iu river and Hii river, flowing into brackish Lakes Nakaumi and Shinji, respectively.

(Received September 17, 2003; Accepted November 13, 2003)

Introduction

Nitrogenous species, such as ammonia, nitrite, nitrate, hydroxylamine (NH₂OH) and nitrous oxide (N₂O), are recycling through microbial processes in the hydrosphere.¹⁻³ NH₂OH has been identified as an intermediate in the nitrogen cycles, such as the oxidation of ammonia to nitrite and the reduction of nitrite to ammonia by several microorganisms in aquatic and sedimentary systems.^{4,5} Furthermore, NH₂OH is often described as an intermediate for producing N₂O. N₂O is one of the greenhouse gases, and is also associated with the reduction of ozone in the stratosphere. Hence, NH₂OH is an important nitrogen species not only for understanding the nitrogen cycle, but also for clarifying the mechanism of N₂O production.

It has been reported that millimolar solutions of NH₂OH are stable for several hours at pH 4.0, but only for 60 min at pH 7.8 in the presence of air.^{6,7} Thus, the direct determination of NH₂OH is difficult in environmental water because of its low concentration and instability.⁸ Several methods, such as spectrophotometric methods^{6,9-11} and a titrimetric method¹² for the determination of NH₂OH, have been reported. The former method is based on the combination of the oxidation of NH₂OH to nitrite with iodine and the usual Griess-Romijn method. This method, however, is subject to interference from nitrite.¹³ The latter method is based on the oxidation of NH₂OH using ferric ammonium sulfate with a copper sulfate catalyst. In this method, Fe(III) was reduced by NH₂OH and the resultant Fe(II) was titrated with potassium dichromate. This method is

applicable in the millimolar range. In general, these methods were employed only in media containing a relatively high concentration of NH₂OH. On the other hand, Marta *et al.* developed a gas chromatographic method for the determination of nanomolar concentrations of NH₂OH by its oxidation to N₂O using Fe(III) ions.¹⁴ However, the yield for the conversion of NH₂OH to N₂O by this method was 50%.

In a previous paper,¹³ we reported the spectrophotometric determination for NH₂OH in environmental water samples. The interference from nitrite in the water sample described above was solved by removing the resultant azo dye from nitrite using a Sep-Pak C₁₈ cartridge prior to the analysis of NH₂OH. Although this method was available for environmental water samples from fresh-water to seawater, the procedure was very tedious. In this paper, we propose a new and simple method for the determination of nanomolar NH₂OH in fresh-water samples by its oxidation to nitrous oxide using hypochlorite as an oxidizing agent. The N₂O produced in this manner was subsequently measured by using a gas chromatograph with an electron-capture detector (ECD).

Experimental

Reagent and apparatus

Reagent solutions were prepared with high-purity water from a Millipore Milli-Q purification system. A standard NH₂OH solution (500 mgN L⁻¹) was prepared by dissolving 0.2481 g of hydroxylammonium chloride in 100 mL of water deoxidized with nitrogen gas. It was freshly prepared for each analysis. A hypochlorite solution was prepared by adding 2.5 mL of commercially available sodium hypochlorite solution (5%

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Fig. 1 Effect of hypochlorite on the oxidation of hydroxylamine to nitrous oxide. The concentrations of NH_2OH added were $3.57 \mu\text{M}$ ($50 \mu\text{gN L}^{-1}$) ($\circ$) and $7.14 \mu\text{M}$ ($100 \mu\text{gN L}^{-1}$) (Δ), respectively.

available chlorine) into 1000 mL of water. A calibration gas, 99.5% grade nitrous oxide (N_2O) was diluted to the desired concentration with 99.9% grade N_2 . Artificial seawater was prepared by dissolving NaCl , MgCl_2 , Na_2SO_4 , CaCl_2 , KCl , NaHCO_3 , KBr , H_3BO_3 , SrCl_2 and NaF in 1 kg of water, according to Lyman and Fleming's procedure.¹⁵

A Shimadzu GC-14B-type gas chromatograph with an electron-capture detector (ECD) was used to determine N_2O . Each pH value in solution was measured with a Horiba F-23 type pH meter.

Condition of gas chromatography

A 2-m long stainless-steel column of 2.6 mm i.d. packed with Unibeads C (80/100 mesh) was used at an oven temperature of 130°C . Each temperature of injection and detector (ECD) was 200 and 300°C , respectively. A carrier gas, 99.9995% grade N_2 , was used at a rate of 50 mL min^{-1} .

Standard procedure

A sample was transferred into a 70-mL glass vial. The vial was sealed by a butyl-rubber stopper and aluminum cap without any head-space, and then 1 mL of a hypochlorite solution was injected to oxidize NH_2OH to N_2O . A small glass ball was included inside for mixing the solution. The head-space technique was used to determine the resultant N_2O in the sample. The head-space of the glass vial was prepared with 99.9% grade N_2 using a gas-tight syringe. After the glass vial was shaken for a few minutes, N_2O in the gas-phase was measured by a gas chromatograph with ECD. The N_2O dissolved in the liquid-phase was calculated by the solubility formula of Weiss and Price.¹⁶ A N_2O blank for each sample was measured without the addition of a hypochlorite solution. This gave the original amount of dissolved N_2O in the samples. NH_2OH was calculated as $\Delta\text{N}_2\text{O}$, which is the difference between the N_2O present in the samples treated and untreated with hypochlorite solution. The detection limit of this gas chromatography for N_2O was $0.1 \mu\text{gN L}^{-1}$.

In field studies, formaldehyde (1% final concentration) was injected into the sample to stop the biological activity for the

Fig. 2 Effect of pH on the oxidation of hydroxylamine ($50 \mu\text{gN L}^{-1}$) to nitrous oxide.

original N_2O analysis. For another sample, 1 mL of a hypochlorite solution was injected to oxidize NH_2OH to N_2O . Both procedures were carried out *in situ* immediately after sampling. The resulting solutions were brought back to the laboratory for gas chromatographic measurements.

Results and Discussion

Optimum condition for the oxidation of hydroxylamine to nitrous oxide

Effect of hypochlorite concentration. The optimum concentration of the hypochlorite solution for the production of N_2O from NH_2OH was examined in 3.57 and $7.14 \mu\text{M}$ (50 and $100 \mu\text{gN L}^{-1}$) of NH_2OH standard solutions, respectively. As shown in Fig. 1, each recovery from 3.57 and $7.14 \mu\text{M}$ of NH_2OH was approximately 100%, when the concentration of hypochlorite solution was higher than 5 and $10 \mu\text{M}$, respectively. Thus, the concentration adopted in the proposed method was $50 \mu\text{M}$ of a hypochlorite solution.

According to the relationship between the added NH_2OH and the produced N_2O by the reaction with hypochlorite (data not shown), the resultant N_2O agreed with the theoretical values calculated using Eq. (1). Thus, the oxidation of NH_2OH to N_2O by hypochlorite was found to proceed stoichiometrically as follows:

Effect of standing time. After adding the hypochlorite solution, the highest recovery was 10 min, indicating that the oxidation reaction of NH_2OH to N_2O by hypochlorite was completed in 10 min. The resultant N_2O was stable for at least 10 days.

Effect of pH. The effect of pH on the oxidation of NH_2OH to N_2O was examined by adjusting the pH of solution with dilute hydrochloric acid and a dilute sodium hydroxide solution. As shown in Fig. 2, the highest, constant recovery was obtained at between pH values of 4 and 8.

Effect of diverse ions. In aquatic environments, nitrogen species other than NH_2OH , such as ammonium, nitrite, nitrate, urea, and amino acid, are present. The influences of such nitrogenous compounds on the determination of NH_2OH were examined in

Fig. 3 Effect of nitrite on the oxidation of hydroxylamine to nitrous oxide. NH_2OH : ○, 50 $\mu\text{gN L}^{-1}$; Δ, 100 $\mu\text{gN L}^{-1}$.

Fig. 4 Effect of salinity in the presence of ammonia (1000 $\mu\text{gN L}^{-1}$) on the oxidation of hydroxylamine to nitrous oxide.

the range of concentration for each species in nature. Figure 3 shows the effect of nitrite on the recovery of NH_2OH to N_2O by the proposed method. The recoveries of NH_2OH were very favorable in the range of 0–200 $\mu\text{gN L}^{-1}$ of nitrite. As reported in a previous paper, nitrite interfered with the determination of NH_2OH using the iodine oxidation technique. Fortunately, nitrite did not interfere with the proposed method. In the range of 0–2000 $\mu\text{gN L}^{-1}$, major nitrogen species, such as ammonium and nitrate, did not interfere with the proposed method (data not shown). In addition, as shown in Table 1, neither several amino acids nor urea interfered with the proposed method.

For the purpose of applying the proposed method to brackish-water and seawater samples, the influence of salinity on the determination of NH_2OH was also examined using artificial seawater and its dilute solutions. Figure 4 shows the effect of the coexisting salinity and ammonia on the recovery of NH_2OH to N_2O . In the presence of ammonia (1000 $\mu\text{gN L}^{-1}$), the recovery of NH_2OH to N_2O decreased with increasing the salinity to more than 1.0 psu. Unfortunately, we found that the proposed method was not applicable to the determination of NH_2OH in brackish water.

Table 1 Effects of amino acids and urea on the recovery of hydroxylamine

Sample ^a	Found/ $\mu\text{gN L}^{-1}$	Recovery, %
NH_2OH (50) only	49.6	—
Glycine (100) + NH_2OH (50)	47.8	96.4
L-Lysine monohydrochloride (100) + NH_2OH (50)	48.2	97.2
L-Glutamic acid (100) + NH_2OH (50)	51.1	103.0
L-Phenylalanine (100) + NH_2OH (50)	48.4	97.6
L- α -Alanine (100) + NH_2OH (50)	47.6	96.0
Urea (100) + NH_2OH (50)	49.3	99.4

a. The values in parentheses are the concentration of each substance in $\mu\text{gN L}^{-1}$.

Table 2 Recovery of hydroxylamine added to fresh-water^a

Sample	Added/ $\mu\text{gN L}^{-1}$	Found/ $\mu\text{gN L}^{-1}$	Recovery, %	RSD ($n = 5$), %
Fresh-water	0	1.9	—	3.6
	20	21.3	97.0	2.1
	50	51.5	99.2	1.4

a. Fresh-water sample was taken from Mabashi river in Matsue, Japan.

Recovery and reproducibility

The recoveries of NH_2OH were examined using an urban river sample in Matsue, to which various amounts of NH_2OH (20 and 50 $\mu\text{gN L}^{-1}$) were added. As shown in Table 2, NH_2OH was recovered quantitatively from the sample solution. The relative standard deviation (RSD) was 1.4–3.6% at concentrations of 2–50 $\mu\text{gN L}^{-1}$.

Application to environmental water

The proposed method was applied to fresh-water samples taken from Iu river and Hii river, flowing into Lakes Nakaumi and Shinji, respectively. For a comparison, the same water samples were determined by using a conventional spectrophotometric method.¹³ For the proposed method, 1 mL of hypochlorite solution was injected to oxidize NH_2OH to N_2O , immediately after sampling, and then the resulting solution was brought back to our laboratory for a gas chromatographic measurement. In the conventional method, acetic acid solution was added to a final concentration of 0.05 M to stabilize NH_2OH in the sample, immediately after sampling. The resulting solution was then brought back to our laboratory for a spectrophotometric measurement. As shown in Table 3, the results obtained by both methods are in good agreement with each other. Thus, the proposed method is satisfactorily applicable to the analysis of fresh-water such as river water and lake water.

It is predictable that NH_2OH is readily oxidized by atmospheric oxygen in aquatic environments because of its extreme lability. As can be seen from Table 3, however, a high concentration of NH_2OH , 50 $\mu\text{gN L}^{-1}$, was found in a reed bed of Hii river. This indicates that the relative kinetics of the two competing reactions, microbial production and autoxidation, may allow the accumulation of NH_2OH in aquatic environments.

Table 3 Analytical results for environmental water samples

Sampling site ^b	Proposed method/ $\mu\text{gN L}^{-1}$	Conventional method ^a / $\mu\text{gN L}^{-1}$
Site A: Iu river		
A-1: Upstream (< 0.1 psu)	0.5	< 1.0
A-2: Upper stream in town (< 0.1 psu)	1.7	1.8
A-3: Middle stream in town (< 0.1 psu)	1.9	1.9
A-4: Down stream in town (< 0.1 psu)	1.3	1.5
A-5: Near estuary (0.30 psu)	2.1	2.0
Site B: Hii river		
B-1: Near estuary (< 0.1 psu)	6.6	6.2
B-2: Estuary (0.18 psu)	0.3	< 1.0
B-3: Reed bed in estuary (0.21 psu)	50.6	49.2

a. Hydroxylamine in environmental water sample was spectrophotometrically determined by the iodine oxidation method with 50-mm glass cell.¹³

b. Water samples of the sites A and B were taken from Iu river and Hii river, Japan, on July 19 and February 5, 2003, respectively.

Conclusion

In this study, a new and simple method for the determination of hydroxylamine in an aquatic environment was developed. Hydroxylamine in a fresh-water sample was oxidized quantitatively to nitrous oxide by using hypochlorite as an oxidizing agent. The nitrous oxide produced in this manner was subsequently measured by a gas chromatograph with an electron-capture detector. The relative standard deviation was 1.4 – 3.6% at hydroxylamine concentrations of 2 – 50 $\mu\text{gN L}^{-1}$.

Acknowledgements

This study was partly supported by a Grant-in-Aid for Scientific Research (No. 14380243) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1. Y. Seike, K. Kondo, H. Hashitani, M. Okumura, K. Fujinaga, and Y. Date, *Jpn. J. Limnol.*, **1990**, *51*, 137.
2. Y. Senga, Y. Seike, K. Mochida, K. Fujinaga, and M. Okumura, *Limnology*, **2001**, *2*, 129.
3. Y. Senga, K. Mochida, N. Okamoto, R. Fukumori, and Y. Seike, *Limnology*, **2002**, *3*, 21.
4. T. Hofman and H. Lees, *Biochem. J.*, **1952**, *54*, 579.
5. M. Tanaka, *Nature*, **1953**, *171*, 1160.
6. M. Fiadeiro, L. Solorzano, and J. D. H. Strickland, *Limnol. Oceanogr.*, **1967**, *12*, 555.
7. J. H. Anderson, *Analyst*, **1954**, *89*, 357.
8. L. R. Pittwell, "Mikrochimica Acta [Wien] 1975 II", **1975**, Springer-Verlag Press.
9. G. Endres and L. Kaufmann, *Ann. Chem.*, **1937**, *530*, 184.
10. R. Novak and P. W. Wilson, *J. Bact.*, **1948**, *55*, 517.
11. T. Z. Csaky, *Acta Chem. Scand.*, **1949**, *2*, 450.
12. K. B. Rao and G. G. Z. Rao, *Anal. Chem.*, **1942**, *64*, 731.
13. R. Fukumori, Y. Senga, M. Okumura, K. Fujinaga, and Y. Seike, *Bunseki Kagaku*, **2003**, *52*, 747.
14. M. T. von Breymann, M. A. de Angells, and L. I. Gordon, *Anal. Chem.*, **1982**, *54*, 1209.
15. J. P. Riley and G. Skirrows, "Chemical Oceanography I", **1965**, Academic Press, New York, 648.
16. R. F. Weiss and B. A. Price, *Mar. Chem.*, **1980**, *8*, 347.