Sensitive Gas Chromatography Detection of Nanomolar Hydroxylamine in Environmental Water by Fe(III) Oxidation

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Nanomolar concentrations of NH₂OH in natural water sources were determined using an Fe³⁺ oxidation method. A pH of 2.35 – 2.50 was used, which was adjusted by adding a chloroacetate buffer. Equal amounts (1.0 mL) of the chloroacetate solution and ferric chloride solution were added to the water sample (70 mL) to oxidize NH₂OH to N₂O. The resulting N₂O in the sample water was then quantified by headspace analysis using a gas chromatograph with an electron-capture detector (ECD), where a limit of detection of 0.2 µgN L^{–1} (14 nmol L^{–1}) was achieved. This method was successfully applied to samples of freshwater, brackish water, and seawater, and despite the various salinities no interfering substances were observed. Furthermore, NH₂OH was successfully detected in samples collected from the Hii River and Lakes Shinji and Nakaumi (Shimane Prefecture, Japan). In addition, the proposed method was also applicable to samples rich in organic substance derived from phytoplankton.

Keywords Brackish water, chloroacetate buffer, estuary, ferric oxidation method, gas chromatography, hydroxylamine, *in situ* method, nitrous oxide

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Introduction

Nitrogenous species, such as ammonia, nitrite, nitrate, hydroxylamine (NH₂OH), and nitrous oxide, are recycled through microbial processes in environmental water.^{1–3} NH₂OH has been identified as an intermediate in nitrogen cycles, including the redox reactions between nitrite and ammonia, in aquatic and sedimentary systems.^{4,5} NH₂OH^{6,7} and hydrazine^{6,8} have attracted particular attention in recent years as an intermediate in anaerobic ammonia oxidation (Anammox). Hence, NH₂OH is an important nitrogen species for understanding the nitrogen cycle.

Millimolar solutions of NH₂OH are stable for several hours at pH 4.0,⁹ but at pH 7.8 in the presence of air, the concentration remains for only 60 min.¹⁰ Thus, the direct determination of NH₂OH is difficult in environmental water because of its low concentration and instability.¹¹ However aquatic chemists and biologists are looking for very low, often nanomolar concentrations of NH₂OH in natural water.

Over the last few decades, several methods for determining nanomolar concentrations of NH₂OH have been developed that employ analytical techniques, such as spectrophotometry,^{12,13} high-performance liquid chromatography (HPLC),¹⁴ and gas

chromatography (GC).^{15,16}

Seike *et al.*¹⁵ have fabricated a simple method for detecting nanomolar NH₂OH in freshwater samples by its oxidation to nitrous oxide (N₂O) using hypochlorite as the oxidizing agent (hypochlorite method). The N₂O produced in this manner was subsequently measured using a gas chromatograph with an electron-capture detector (ECD). Kato *et al.*¹⁶ modified this hypochlorite method, developing a quantitative method that could be applied to brackish and sea water, as well as freshwater samples. This method exhibited a limit of detection of 0.2 µgN L^{–1} (14 nmol L^{–1}) for detecting NH₂OH. However, we have noticed one drawback for this method,^{15,16} that is, in the case of samples rich in organic matter derived from phytoplankton, the amount of NH₂OH detected might be underestimated.

Another method was proposed by von Breymann *et al.*¹⁷ that employed gas chromatography to detect NH₂OH by its oxidation to N₂O, with Fe³⁺ acting as the oxidizing agent (Fe(III) method). However, only 50% conversion of NH₂OH to N₂O was achieved. Butler and Gordon¹⁸ modified this Fe(III) method,¹⁷ and they reported conversions of NH₂OH to N₂O that reached 80% (40 – 83%) by adjusting the pH to 2.9 – 3.0 using an acetate buffer. The assumed reaction for these analyses is as follows:

where N₂O is the sole nitrogenous end-product. In this study, we further modified the Fe(III) method¹⁸ and attained complete

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Fig. 1 Standard quantitative procedure.

conversion of NH_2OH to N_2O . We also examined the effect of organic substances that were provided by aquatic organisms for the determination of NH_2OH in natural water using our modified Fe(III) method.

Experimental

Reagents and chemicals

High-purity reagents (Wako, Special Class) were used in all cases. Milli-Q water (Millipore) was used for all reagent preparations.

An NH_2OH standard solution (500 mgN L^{-1}) was prepared by dissolving 0.2481 g of hydroxylamine hydrochloride in deoxygenated water and diluting it to 100 mL. Further, suitable dilutions were made at the time of use. Fresh reagents were prepared for each experiment.

A calibration gas, 99.5% N_2O , was diluted to the desired concentration with 99.99995% grade N_2 .

A ferric chloride solution (105 mmol L^{-1}) was prepared by dissolving 2.838 g of ferric chloride in 100 mL of water.

A chloroacetate buffer solution (pH 2.4) was prepared by dissolving 13.22 g of chloroacetic acid and 1.692 g of potassium hydroxide in 100 mL of water.

Artificial seawater (ASW) was prepared¹⁹ by dissolving 23.4 g of NaCl, 4.981 g of MgCl_2 , 3.917 g of Na_2SO_4 , 1.102 g of CaCl_2 , 0.664 g of KCl, 0.192 g of NaHCO_3 , 0.096 g of KBr, 0.026 g of H_3BO_3 , 0.024 g of SrCl_2 , and 0.003 g of NaF in 1 kg of water.

Apparatus

A Shimadzu GC-14B-type gas chromatograph with an ECD was used to detect N_2O . The pH of each solution was measured with a Horiba F-23 pH meter.

Conditions of gas chromatography

A stainless-steel column (length, 2 m; i.d., 2.6 mm) that was packed with Unibeads C (mesh 60/80, GL Sciences) was used at an oven temperature of 130°C . The temperatures of the injector and detector (ECD) were 200 and 300°C , respectively. The 99.99995%-grade N_2 carrier gas was used at a rate of 50 mL min^{-1} . The water vapor in the sample was trapped with a sequential precolumn packed with CaSO_4 before entering the Unibeads C column.²⁰

Standard procedure

Each water sample was transferred into a 70-mL brown glass vial with glass beads that would mix the solution. The vial was capped with butyl rubber and an aluminum seal without any head-space to prevent air intrusion. Then, 1.0 mL of the chloroacetate buffer solution (pH = 2.4) and 1.0 mL of the 105 mmol L^{-1} ferric chloride solution were injected (Fig. 1) to oxidize NH_2OH to N_2O at room temperature. A headspace technique was used to quantify the N_2O generated. Then, 40 mL of N_2 gas (99.99995% purity) was added to the vial using a magnum syringe for headspace analysis. After shaking the vial for several minutes, 0.2 mL of the headspace gas was injected into the gas chromatograph to measure the N_2O in the headspace. The N_2O concentration in the liquid phase was calculated using the Weiss and Price formula.²¹ For the blank, we performed each measurement with the addition of a buffer solution and ferric chloride solution against sample water free from NH_2OH . The NH_2OH concentration was obtained by subtracting the N_2O blank from the N_2O signal generated by the buffer and a ferric chloride solutions. The detection limit of N_2O by gas chromatography was $0.2 \text{ } \mu\text{gN L}^{-1}$.

For the original N_2O analysis in field studies, only formaldehyde (1% final concentration) was injected into the water samples to stop biological activity. This provided the original amount of dissolved N_2O in the samples. For the NH_2OH samples, 1.0 mL of a buffer solution and 1.0 mL of a ferric chloride solution were injected to oxidize NH_2OH to N_2O . The resulting solutions were brought back to the laboratory for gas chromatographic measurements.

Results and Discussion

Optimum conditions for oxidizing hydroxylamine to nitrous oxide

For the purpose of applying the proposed method to seawater as well as freshwater, the influence of salinity on the determination of NH_2OH was also examined using artificial seawater.

Effect of pH

Samples were prepared with H^+ concentrations of $0.1 - 0.01 \text{ mol L}^{-1}$ and pH values of 2 – 8. The ferric chloride solution had been added to both the freshwater (MQW) samples and the artificial seawater (ASW, 35‰) samples, after which each of a dilute sodium hydroxide solution and a dilute sulfuric acid solution was added to the MQW samples and to the ASW

Fig. 2 Effect of the pH on the oxidation of NH_2OH ($50 \mu\text{gN L}^{-1}$) to N_2O in sample solutions prepared with freshwater (MQW, ●) and artificial seawater (35‰ ASW, ○). 1.0 mL of the 105 mmol L^{-1} ferric chloride solution was injected. These data shown are mean values ($n = 2$).

Fig. 3 Effect of the amount of buffer solution added on the oxidation of NH_2OH ($50 \mu\text{gN L}^{-1}$) to N_2O in sample solutions prepared with MQW (●) and ASW (○). 1.0 mL of the 105 mmol L^{-1} ferric chloride solution was injected. These data shown are mean values ($n = 2$).

samples. The highest, constant value was obtained between a pH of 2.00 and 2.85 (Fig. 2), and we also attained a recovery of approximately 100%. Thus, we were able to quantitatively recover NH_2OH as N_2O . Our optimum pH range (2.00 – 2.85) was different from that (2.8 – 3.5) determined by Butler and Gordon.¹⁸ However, we obtained similar results, where our recovery of NH_2OH as N_2O was 71% at a pH of 3.0, while Butler and Gordon achieved their maximum conversion of 80% at a pH of 2.9 – 3.0.

Effect of buffer solution addition

Butler and Gordon¹⁸ reported low recoveries (40 – 83%) of NH_2OH as N_2O when acidifying their samples to a pH of 2.9 – 3.0 using an acetate solution as their buffer. Our study employed the optimum pH range of 2.00 – 2.85 (Fig. 2); therefore, we chose not an acetate solution, but a chloroacetate solution (pH 2.4) as our buffering agent to adjust to a pH of 2.35 – 2.50. To confirm its suitability, we examined the effects of the amount of chloroacetate solution added to the sample water on the conversion of NH_2OH to N_2O (Fig. 3). We found that adding 0.5 – 3.0 mL of the chloroacetate solution to 70 mL of the sample solution adjusted the pH of 2.35 – 2.50 and achieved quantitative recoveries of NH_2OH from N_2O . From these results, we chose to add 1.0 mL of a chloroacetate solution to the samples in subsequent experiments.

Effect of the Fe^{3+} concentration

NH_2OH standard solutions with concentrations of 50 and $20 \mu\text{gN L}^{-1}$ (3.57 and $1.43 \mu\text{mol L}^{-1}$) were tested against MQW and ASW (35‰) samples, respectively, to determine the effect

Fig. 4 Effect of the Fe^{3+} concentration on the oxidation of NH_2OH (20 and $50 \mu\text{gN L}^{-1}$) to N_2O in sample solutions prepared with MQW and ASW. 1.0 mL of the chloroacetate buffer solution (pH = 2.4) was injected. ▲, NH_2OH ($20 \mu\text{gN L}^{-1}$) in MQW; △, NH_2OH ($20 \mu\text{gN L}^{-1}$) in ASW; ●, NH_2OH ($50 \mu\text{gN L}^{-1}$) in MQW; ○, NH_2OH ($50 \mu\text{gN L}^{-1}$) in ASW. These data shown are mean values ($n = 2$).

of the Fe^{3+} concentration on the oxidation of NH_2OH to N_2O . These experiments were each conducted at a pH of 2.35 – 2.50, for 2 h, at 25°C . When the concentration of Fe^{3+} was in the range of 1.2 – 2.5 mmol L^{-1} , the recovery of NH_2OH from both the 50 and $20 \mu\text{gN L}^{-1}$ solutions was approximately 100% (Fig. 4). Therefore, the ferric chloride concentration adopted for our proposed method was 1.5 mmol L^{-1} , which corresponds to the final concentration when 1.0 mL of 105 mmol L^{-1} ferric chloride solution was added to 70 mL of the water sample.

Effect of the standing time

After adding a ferric chloride solution, the highest recovery was obtained after 1 h, where it remained constant, indicating that the oxidation reaction of NH_2OH to N_2O by the Fe(III) solution was completed in about 1 h. The resultant N_2O was stable for at least 10 days (data not shown). For this study, we adopted 2 h as the standing time.

Effects of diverse ions

The seawater contains Na^+ , Mg^{2+} , Ca^{2+} , K^+ , Cl^- , SO_4^{2-} , and HCO_3^- at extremely high levels: such ions universally exist in fresh river and lake waters only at low levels. As can be seen from Figs. 2 – 4, NH_2OH was able to be determined even in seawater samples.

Nitrogen species other than NH_2OH , such as NH_4^+ , NO_2^- , NO_3^- , urea, and amino acids, are present in environmental water. We investigated the effects of these substances on the determination of NH_2OH using our improved Fe(III) method. No interference was observed by NH_4^+ , NO_2^- , or NO_3^- over the range of 0 to $1000 \mu\text{gN L}^{-1}$, and neither urea nor several amino acids interfered with concentrations over the range of 0 to $100 \mu\text{gN L}^{-1}$ (Table 1).

However, $500 \mu\text{gN L}^{-1}$ or greater of nitrite has been observed to interfere with NH_2OH detection by the hypochlorite method.¹⁶ However, no interference due to nitrite at concentrations up to $1000 \mu\text{gN L}^{-1}$ was observed in our proposed method.

Effect of organic matter (Chl-a)

When using the hypochlorite method^{15,16} to measure samples that contained organic substances (especially phytoplankton), it was suggested that some of the measurements could be underestimated (unpublished data). Therefore, we investigated the NH_2OH recovery of the hypochlorite method and our improved Fe(III) method using the same water samples that contained phytoplankton (Fig. 5). The water sample that contained phytoplankton was taken in epilimnion of brackish

Table 1 Effects of ammonium, nitrite, nitrate, amino acids, and urea on the recovery of NH_2OH ($50 \mu\text{gN L}^{-1}$) as N_2O using the improved Fe(III) method

Sample ^a	Found/ $\mu\text{gN L}^{-1}$	Recovery, %	RSD ($n = 3$), %
NH_2OH (50) only	49.5	99.0	0.7
Ammonium (1000) + NH_2OH (50)	49.8	99.7	0.1
Nitrite (1000) + NH_2OH (50)	49.5	99.0	0.8
Nitrate (1000) + NH_2OH (50)	49.7	99.4	0.7
Glycine (100) + NH_2OH (50)	49.2	98.4	0.2
L-Glutamic acid (100) + NH_2OH (50)	49.5	99.1	0.3
L-Phenylalanine (100) + NH_2OH (50)	49.3	98.5	0.5
L- α -Alanine (100) + NH_2OH (50)	49.3	98.7	0.2
L-Lysine monohydrochloride (100) + NH_2OH (50)	49.5	99.1	0.5
Urea (100) + NH_2OH (50)	49.5	99.0	0.4

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

Fig. 5 Effect of an organic substance (Chl-*a*) on the oxidation of NH_2OH ($50 \mu\text{gN L}^{-1}$) to N_2O by the hypochlorite method (●) and the improved Fe(III) method (○). 1.0 mL of the chloroacetate buffer solution (pH = 2.4) and 1.0 mL of the 105 mmol L^{-1} ferric chloride solution were injected.

Table 2 Recovery of NH_2OH (20 and $50 \mu\text{gN L}^{-1}$) added to the natural freshwater and brackish-water samples using the improved Fe(III) method

Sample	Added/ $\mu\text{gN L}^{-1}$	Found/ $\mu\text{gN L}^{-1}$	Recovery		RSD ($n = 5$), %
			$\mu\text{gN L}^{-1}$	%	
Hii river (Freshwater)	0	0.6	—	—	—
	20	21.3	20.6	103	2.1
	50	51.5	50.9	102	2.1
Lake Shinji (Salinity, 8.0‰)	0	0.4	—	—	—
	20	20.2	19.8	99	1.3
	50	50.9	50.6	101	1.2
Lake Nakaumi (Salinity, 24.8‰)	0	0.6	—	—	—
	20	20.5	19.9	99	2.1
	50	51.4	50.9	102	1.1

Lake Shinji. The suspended substance (mainly phytoplankton) in water samples was collected on a membrane filter ($0.5 \mu\text{m}$), and the Chl-*a* concentration in each sample was adjusted accordingly using the suspension on the filter and its filtered water. Since the filtered water was used to dilute each sample, the salinity in all samples was constant. The recovery of the hypochlorite method decreased with increasing the Chl-*a* concentration, while that of our proposed method was high and remained constantly regardless of Chl-*a* concentration. The decrease in the recovery of the hypochlorite method was due to a lack of hypochlorite, resulting from phytoplankton decomposition. Thus, in the case of samples containing high amounts of various kinds of organic substances, that come from phytoplankton, it was necessary to add an excess amount of hypochlorite compared with the conventional method. Our improved Fe(III) method did not exhibit a decrease in the recovery because the weak oxidation of Fe(III) did not contribute to the decomposition of phytoplankton. Based on these results, a suitable method must be selected for testing in various environments.

Calibration curve, recovery and reproducibility

When a calibration curve was drawn in the range of 0 to 100 $\mu\text{gN L}^{-1}$, a good result with a high linearity ($y = 0.001x + 0.0975$ and $R^2 = 0.9991$) was obtained.

To confirm the recovery of NH_2OH as N_2O by the proposed method, we performed experiments in which 20 and 50 $\mu\text{gN L}^{-1}$ solutions of NH_2OH were added to freshwater and brackish-

Fig. 6 Vertical distributions of NH_2OH , N_2O , and Chl-*a* present in samples from the Sanbe-dam reservoir (collected September 25, 2019). ●, NH_2OH ; ○, N_2O ; △, Chl-*a*.

water samples, respectively. NH_2OH was quantitatively recovered from the water samples in a range of 99 to 103% with a relative standard deviation (RSD) of 1.1 to 2.1% (Table 2).

Application to environmental water samples

The proposed method was applied to a water sample from the Sanbe-dam reservoir, collected on September 25, 2019. Sample water was taken with a Kitahara-type sampler and transferred to a brown glass vial (70 mL). Then, 1.0 mL of a buffer solution (pH 2.4) and 1.0 mL of a ferric chloride solution (105 mmol L⁻¹) were added to determine the amount of NH₂OH as N₂O. For the original N₂O analysis, formaldehyde (1% final concentration) was added to stop biological activity. These procedures were performed *in situ*, and then the samples were transported to the laboratory. The headspace technique using gas chromatography was then employed to measure the N₂O concentration. The vertical distributions of NH₂OH, N₂O, and Chl-*a* are shown in Fig. 6. An interesting phenomenon was observed, where NH₂OH and Chl-*a* exhibited a similar behavior. It seems likely that this NH₂OH might be derived from phytoplankton.

Conclusions

We developed a method for determining nanomolar NH₂OH concentrations in environmental water based on Fe³⁺ oxidation. To detect the NH₂OH concentration, a chloroacetate solution (buffer) and a ferric chloride solution (oxidant) were added to the sample solutions to oxidize NH₂OH to N₂O, which was then quantified by headspace analysis using a gas chromatograph equipped with an ECD. Our method exhibited a high NH₂OH recovery and good reproducibility when testing various samples from freshwater, brackish water, and seawater sources in Japan. In addition, it was also applicable to samples rich in organic substances, derived from phytoplankton.

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