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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conversion of NH₂OH to N₂O. We also examined the effect of organic substances that were provided by aquatic organisms for the determination of NH₂OH 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 NH₂OH standard solution (500 mg N 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% N₂O, was diluted to the desired concentration with 99.99995% grade N₂.

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 prepared by dissolving 23.4 g of NaCl, 4.981 g of MgCl₂, 3.917 g of Na₂SO₄, 1.102 g of CaCl₂, 0.664 g of KCl, 0.192 g of NaHCO₃, 0.096 g of KBr, 0.026 g of H₂BO₃, 0.024 g of SrCl₂, 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 N₂O. 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 N₂ 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 CaSO₄ before entering the Unibeads C column.

**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 NH₂OH to N₂O at room temperature. A headspace technique was used to quantify the N₂O generated. Then, 40 mL of N₂ 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 N₂O in the headspace. The N₂O concentration in the liquid phase was calculated using the Weiss and Price formula. For the blank, we performed each measurement with the addition of a buffer solution and ferric chloride solution against sample water free from NH₂OH. The NH₂OH concentration was obtained by subtracting the N₂O blank from the N₂O signal generated by the buffer and a ferric chloride solutions. The detection limit of N₂O by gas chromatography was 0.2 μg N L⁻¹.

**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 NH₂OH was also examined using artificial seawater.

**Effect of pH**

Samples were prepared with H⁺ concentrations of 0.1 - 0.01 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
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 NH₄OH as N₂O. Our optimum pH range (pH = 2.4) was different from that (2.8 – 3.5) determined by Butler and Gordon.18 However, we obtained similar results, where our recovery of NH₂OH as N₂O was 71% at a pH of 3.0, while Butler and Gordon achieved their maximum conversion of 80% approximately 100%. Thus, we were able to quantitatively recover NH₂OH as N₂O. Our optimum pH range (2.00 – 2.85) was obtained between a pH of 2.35 – 2.50 and we also attained a recovery of NH₂OH from both the 50 and 20 μgN 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 NH₂OH to N₂O by the Fe(III) solution was completed in about 1 h. The resultant N₂O 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⁺, Mg²⁺, Ca²⁺, K⁺, Cl⁻, SO₄²⁻, and HCO₃⁻ 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, NH₂OH was able to be determined even in seawater samples.

Nitrogen species other than NH₂OH, such as NH₄⁺, NO₂⁻, NO₃⁻, urea, and amino acids, are present in environmental water. We investigated the effects of these substances on the determination of NH₂OH using our improved Fe(III) method. No interference was observed by NH₄⁺, NO₂⁻, or NO₃⁻ over the range of 0 to 1000 μgN L⁻¹, and neither urea nor several amino acids interfered with concentrations over the range of 0 to 100 μgN L⁻¹ (Table 1).

However, 500 μgN L⁻¹ or greater of nitrite has been observed to interfere with NH₂OH detection by the hypochlorite method.16 However, no interference due to nitrate at concentrations up to 1000 μgN L⁻¹ was observed in our proposed method.

Effect of organic matter (Chl-a)
When using the hypochlorite method15,16 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 NH₂OH 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
Lake Shinji. The suspended substance (mainly phytoplankton) in water samples was collected on a membrane filter (0.5 μm), 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 μgN L⁻¹, a good result with a high linearity (y = 0.001x + 0.0975 and R² = 0.9991) was obtained.

To confirm the recovery of NH₂OH as N₂O by the proposed method, we performed experiments in which 20 and 50 μgN L⁻¹ solutions of NH₂OH were added to freshwater and brackish-water samples, respectively. NH₂OH 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).

| Sample | Added/μgN L⁻¹ | Found/μgN L⁻¹ | Recovery, % RSD (n = 5), % |
|--------|--------------|--------------|---------------------------|
| Hii river (Freshwater) | 0 | 0.6 | — | — |
| 50 | 21.3 | 20.6 | 103 | 2.1 |
| Lake Shinji (Salinity, 8.0%) | 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 |

**Fig. 5** Effect of an organic substance (Chl-a) on the oxidation of NH₂OH (50 μgN L⁻¹) to N₂O 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.

**Fig. 6** Vertical distributions of NH₂OH, N₂O, and Chl-a present in samples from the Sanbe-dam reservoir (collected September 25, 2019). ● NH₂OH; ○ N₂O; —, Chl-a.
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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