**Introduction**

Hydrazine is a highly reactive substance used in analysis, metallic coating, photographic chemicals, antioxidants, pesticides, and production of plastic. In recent years, hydrazine has been reported as an intermediate formed during anaerobic ammonium oxidation (Anammox)-type denitrifying reaction. Quantifying the hydrazine produced leads to a better understanding of the Anammox reaction. Hydrazine content is conventionally determined by voltammetry, coulometry, gas chromatography, fluorescence spectroscopy, spectrophotometry, and titration. Quantification of low concentrations of hydrazine in a water sample is usually done by spectrophotometry, with a typical assay using p-dimethylaminobenzaldehyde (p-DMAB) colorimetry.

A simple and rapid in situ method for the determination of hydrazine based on the concentration of aldazine compound formed by the reaction of hydrazine with p-dimethylaminobenzaldehyde was developed. This method was based on solid-phase extraction using a Sep-Pak C18 cartridge, followed by the quantification of hydrazine using a spectrophotometric method. To a sample solution of environmental water, p-dimethylaminobenzaldehyde solution was added to form aldazine by the reaction with hydrazine. The solution was passed through a Sep-Pak C18 cartridge for the adsorption of aldazine. In the laboratory, the aldazine adsorbed on the Sep-Pak C18 cartridge was eluted by passing a hydrochloric acid–ethanol (1:10) solution through the cartridge, and the color intensity of the solution was measured at 457 nm. The limit of detection for the new method was 0.2 mgN L⁻¹ of hydrazine. The determination of hydrazine in solution was not influenced even by hydrogen sulfide and organic matter. This method was then applied to the brackish water of Lake Nakaumi in the eastern area of Shimane Prefecture, Japan. This method was used to determine hydrazine in freshwater, seawater and wastewater.

**Keywords** Environmental water, hydrazine, in situ method, preconcentration, Sep-Pak C18 cartridge, spectrophotometry

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Experimental

Reagent and apparatus
A standard hydrazine solution (100 mgN L⁻¹) was prepared by dissolving hydrazine dihydrochloride (0.318 g) in hydrochloric acid (12 M, 10 mL) and water (20 mL), and made up to 1000 mL with water. Working standards were prepared by serial dilution of the stock solution with hydrochloric acid (1:99). The standard solution was remade once a month.

A p-DMAB solution was prepared by dissolving p-DMAB (2.0 g) in methanol (200 mL) and hydrochloric acid (12 M, 20 mL), and was stored in a dark brownish glass bottle. A hydrochloric acid (1:99) solution was made by making up hydrochloric acid (6 M, 20 mL) to 1000 mL with water. A hydrochloric acid–ethanol (1:10) solution was freshly prepared for each analysis by mixing hydrochloric acid (12 M, 10 mL) with 100 mL of ethanol. A standard urea solution (100 mgN L⁻¹) was prepared by dissolving urea (0.858 g) in water to 1000 mL. A standard hydrogen sulfide solution (100 mgS L⁻¹) was prepared by dissolving sodium sulfide (0.76 g) in water, then it was neutralized with hydrochloric acid.

All high-purity reagents (Suprapur, Wako Co.) were used. In all analytical procedures, Milli-Q water (Millipore Co.) was used.

A peristaltic pump (SMP-23, EYELA Co.) was used for quantitative liquid transfer. A Sep-Pak C18 cartridge (Waters Co.) packed with C18-bonded silica gel was used as the solid-phase adsorbent. A Shimadzu UV-1800 spectrophotometer with a 50-mm glass cell was used to determine hydrazine.

Standard procedure
Prior to use, the Sep-Pak C18 cartridge was rinsed successively with ethanol (10 mL), 50% ethanol (8 mL), and water (10 mL), at a flow rate of 10 mL min⁻¹. The conditioned Sep-Pak C18 cartridge was kept tightly closed with a silicon stopper and rubber plug to prevent drying. The cartridge was connected to a filter holder with a 0.2 μm-Nuclepore filter (25 mm in diameter).

The sample solution was taken in a 50-mL graduated plastic syringe and passed through the cartridge via the filter. To the sample in the syringe, 6 mL of p-DMAB solution was added through the three-way trap (Terumo Co.) with a 10-mL graduated syringe, so that it reacts with hydrazine to form aldazine. After 10 min, the resulting yellowish solution was passed through the cartridge at a flow rate of 8 mL min⁻¹. In the laboratory, the aldazine adsorbed on the Sep-Pak C18 cartridge was eluted by passing 8 mL of the hydrochloric acid-ethanol (1:10) solution through the cartridge at a flow rate of 10 mL min⁻¹. The absorbance of the eluent was measured at 457 nm.

Results and Discussion

Absorption spectra
The absorption spectra of aldazine formed in the eluent measured against water is shown in Fig. 2. Aldazine has an absorption maximum at 457 nm. At this wavelength, the absorbance of reagent blank is negligible. Therefore, all absorbance measurements were carried out at 457 nm in this study.

Collection on Sep-Pak C18
The collection of aldazine from hydrazine on the Sep-Pak C18 was examined by passing a solution containing 10 μgN L⁻¹ as hydrazine according to the standard procedure. The formed aldazine was quantitatively adsorbed on the Sep-Pak C18 cartridge. As determined by a breakthrough method, the cartridge was found to have an adequate adsorption capacity, and was able to collect aldazine corresponding to at least 2.0 μgN of hydrazine.

Sep-Pak C18 cartridges that had adsorbed aldazine were stored in a dark place at 25°C and analyzed over a period of two days to examine the stability of aldazine on the cartridges. The aldazine was stable on the Sep-Pak C18 for at least 12 h (Fig. 3).

Elution
The elution of aldazine corresponding to 0.25 μgN of hydrazine from the Sep-Pak C18 was examined using ethanol and acetone. Acetone eluted the adsorbed aldazine, but the yellowish eluate soon exhibited a color fading reaction. Although ethanol almost completely eluted the aldazine from the Sep-Pak C18, a precipitate was formed in the eluate. The addition of a 12 M hydrochloric acid solution to ethanol improved the elution of aldazine by dissolution of the precipitate. The formation of a precipitate was perfectly avoided by using an eluent containing more than 5 – 15% (v/v) of a 12 M hydrochloric acid in ethanol. As shown in Fig. 4, the aldazine was sufficiently eluted with a 12 M hydrochloric acid (15 – 5%) in ethanol (85 – 95%). In this study, a mixture of ethanol and a 12 M hydrochloric acid (10:1, v/v) was used as an eluent.
The absorbance of an eluate containing the aldazine remained constant for at least 2 h.

**Effect of flow rate**

To examine the effect of flow rate on the adsorption of aldazine, 50 mL of a 10 μgN L⁻¹ aldazine solution was passed through the Sep-Pak C18, and the aldazine concentration in the effluent passed through the cartridge was investigated. Aldazine was quantitatively adsorbed when the flow rate was 2 – 9 mL min⁻¹. Therefore, for *in situ* operation, there is no problem if the flow rate is somewhat changed during the suction with syringe. A flow rate of 8 mL min⁻¹ for the water sample at the time could be operated manually. The aldazine adsorbed on the cartridge was readily eluted with 10 mL of the eluent within 1 min.

**Calibration graph and precision**

The calibration graph was linear for up to 1.0 μgN aldazine in 8 mL of the eluent. The absorbance per 5.0 μgN L⁻¹ of aldazine was 0.245 at 457 nm. The amount of aldazine corresponding to that of hydrazine for up to 1.0 μgN adsorbed on a Sep-Pak C18 cartridge was quantitatively eluted with 8 mL of the eluent. The relative standard deviation for the observed value of 5.0 μgN L⁻¹ was 3.6%.

**Suppression of interfering substances**

The compound *p*-DMAB, used as a color former, is known to react with urea and hydrogen sulfide present in environmental water. Therefore, we investigated their effect on the results of our analysis. Standard urea (100 mgN L⁻¹) and hydrogen sulfide (100 mgS L⁻¹) solutions were prepared by diluting them with hydrochloric acid (1:99). Figure 5 shows the effects of the urea and hydrogen sulfide on the resulting color sample (Sep-Pak passed through before), effluent, and eluent used in this method. Urea and hydrogen sulfide caused interference at the measured absorption wavelength of the color samples at around 457 nm, and the effluent and eluent both showed absorption characteristics similar to that of the blank, at around 457 nm. Therefore, interference caused by urea and hydrogen sulfide in the determination of hydrazine could be suppressed by using a Sep-Pak cartridge. The proposed method can also be used to determine the hydrazine concentration in environmental waters containing high concentrations of urea and hydrogen sulfide by using a Sep-Pak C18.

In addition, for the purpose of applying the proposed method to brackish and seawater samples, the influence of the salinity on the determination of hydrazine was also examined using artificial seawater and its dilute solution. The artificial seawater was according to Lyman and Flaming’s procedure. Seawater contains Na⁺, Mg²⁺, Ca²⁺, K⁺, Cl⁻, and SO₄²⁻ at extremely high levels (the order of g/L) and such ions universally exist in fresh river and lake waters only in low levels (the order of mg/L). The determination of hydrazine in solution was not influenced even by high salinity (34‰), such as that of seawater.

**Organic matter removal**

The effect of dissolved organic matter found in environmental water on the proposed method was investigated. Substances such as organic matter that were adsorbed to the Sep-Pak C18 were removed prior to using the environmental water. A 50-mL sample of wastewater was introduced into a 50-mL graduated syringe as a sample solution, and then the spectra of the filtrate obtained with a 0.2-μm-Nuclepore filter and the effluent passed through the Sep-Pak C18 via the filter were measured (Fig. 6). Although the filtration only results in a disturbance showing a large absorption near 457 nm, an absorption wavelength was not observed for the effluent passed through the cartridge, showing that the interference was prevented by using the Sep-Pak C18.
The use of Sep-Pak C18 was effective for the removal of organic matter in waste water.

Recovery and reproducibility
Recoveries of hydrazine were examined using various environmental water samples with added hydrazine (10 μgN L⁻¹). As shown in Table 1, hydrazine was quantitatively recovered from the sample solutions with a standard deviation (RSD) of 2.0 – 3.0%. The recovery rate of hydrazine was in the range of 92 to 98%.

Application to environmental water
The proposed method was applied to water samples from the brackish Lake Nakaumi, Japan, on August 21, 2014. Each water sample was divided into two aliquots. One aliquot was used for the proposed method, and the other for a conventional method, which was employed for a comparison with the proposed method. After obtaining the samples using a Kitahara-type water sampler, 50 mL aliquots were passed through a short silicon tube connecting the sampler and the graduated syringe without any contact with air. For the proposed method, all operations for hydrazine collection using the Sep-Pak C18 were carried out on a small boat on the lake. The Sep-Pak C18 was brought back to our laboratory for elution and spectrophotometric analysis. For the conventional method, a color development of sample solutions as aldazine was made on the boat as well as the operation for the proposed method. The colored sample solutions were transported back to the laboratory to spectrophotometrically analyze the absorbance within 5 h of sample collection, because the colored solutions were stable for about 12 h. The proposed method could be applied to water samples from Lake Nakaumi, but the conventional method could not be applied. This is the reason that the hydrazine concentration in all samples from Lake Nakaumi were lower than the detection limit (2.0 μgN L⁻¹) of the conventional method (Fig. 7).

In addition, water samples for measurement of ammonium, nitrite, and nitrate were collected in polyethylene bottles, filtered, and analyzed immediately after being brought back to the laboratory. Vertical distributions of the hydrazine, ammonium, nitrite, and nitrate are given in Fig. 7. Hydrazine concentration started increasing after a 3-m depth, and reached a high value of 1.0 μgN L⁻¹ at a depth of 5 – 6.5 m. Ammonium and nitrite also reached higher concentrations with increasing depth. Ammonium was supplied by release from the sediments at the bottom of the lake, and nitrite has been reported to accumulate owing to the oxygen supplied by the inflow of seawater from the Sea of Japan and photoinhibition of nitrite-oxidizing bacteria.17) Ammonium and nitrite are abundant near the bottom layer of Lake Nakaumi, and are substrates of the Anammox reaction. The hydrazine intermediate of the Anammox reaction was detected at high concentrations.

Conclusions
A simple and rapid method for the in situ separation and preconcentration of hydrazine in environmental waters has been developed, based on solid-phase extraction using a Sep-Pak C18 cartridge. The proposed method involved the reaction of hydrazine with p-DMAB in situ to form aldazine, and the solid-phase extraction of the formed aldazine with the Sep-Pak C18 cartridge. The Sep-Pak C18 cartridges were brought back to the laboratory for elution using a hydrochloric acid-ethanol (1:10)
solution and for spectrophotometric analysis. Using the proposed method, 6.25 times aldazine can be recovered compared to that obtained using conventional methods. Our method can also be applied to fresh water, brackish water, and wastewater after removing organic matter in those samples by using the Sep-Pak C18 cartridge.

The proposed method was used for analyzing environmental water samples from Lake Nakaumi, and it was found that the bottom layer was rich in hydrazine, ammonium, and nitrite, suggesting the occurrence of the Anammox reaction.

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References