Flow Injection Analysis of Nitrite Nitrogen in Seawater Introducing Standard Addition Method

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Abstract

A flow injection analysis of nitrite nitrogen (N-NO_2^-) in the samples which contain relatively high degrees of salt is described. The N-NO_2^- was optically detected by use of Griess method. Five pseudo-seawater samples containing 40 μg L^{-1} N-NO_2^- with 0, 1, 2, 3, or 4% sodium chloride (NaCl) were tested using two quantitative analysis approaches. In an absolute calibration method, approximately 30% of positive errors were found in the presence of NaCl. On the other hand, the errors fell within the range of −2.6 to +7.3% by introducing a standard addition method. The latter method has successfully been applied to the determination of nitrite nitrogen in real seawater and estuary water.

Keywords Nitrite, seawater, flow injection analysis, standard addition method

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1. Introduction

Nitrogen compounds are ubiquitous in the environment [1]. Excessive inflow of nitrite (NO_2^-) into estuary causes eutrophication, resulting in red and blue tides, which damage fisheries and contaminate shorelines [2]. In addition, the NO_2^- taken into the body causes an oxygen deficiency state (methemoglobinemia), which can result in death [3]. The United State Environmental Protection Agency has set a maximum contaminant level for nitrite nitrogen (N-NO_2^-) in drinking water at 1 mg L^{-1} [4]. In 2014, the Ministry of Health, Labour and Welfare set a standard value of Drinking Water Quality Standards in Japan for N-NO_2^- to 0.04 mg L^{-1} [5].

Griess method [6] based on the coupling of diazotized sulfanilamide and *N*-(1-Naphthyl)ethylenediamine [7] is one of the most popular approaches for NO_2^- determination. Flow injection analysis (FIA) described by Ruzicka and Hansen in 1975 [8] has been proven to be very useful in numerous literature. Several papers [9-11] described the FIA based on the Griess reaction for the determination of NO_2^- in natural waters. However, the samples such as seawater and estuary water, which contain relatively high degrees of salt would generate errors in the Griess reaction based determination [12]. The simplest approach for suppressing the matrix interference is matrix matching method [13]. However, the salt level in the samples would vary according to the sampling seasons, locations, and times, *etc*. Standard addition method is useful when it is difficult or impossible to duplicate the sample matrix.

In this study, we investigated the FIA introducing standard addition method so as to improve the accuracy of N-NO_2^- determination in seawater. The pseudo-seawater and real seawaters were tested as the samples which contain relatively high degrees of salt. The data comparing the standard addition method and the absolute calibration method are presented in this paper.

2. Experimental

2.1. Reagents, pseudo-seawater and real seawater samples

All reagents used in this study were of analytical grade and were used without further purification. Sulfanilamide, *N*-(1-Naphthyl)ethylenediamine dihydrochloride, and sodium chloride (NaCl) were purchased from Kanto Chemical Co., Inc. Hydrochloric acid and sodium nitrite were purchased from Nacalai Tesque, Inc. Sartorius arium 611 DI grade deionized water (>18 MΩ cm) was used throughout.

Sodium chloride is the dominant salt component in seawater. Pseudo-seawater samples were, therefore, prepared by adding NaCl to N-NO_2^- standard solution. Real seawater samples were collected from the mouth of the Imagire River, Tokushima, Japan and the coast of the Uwa Sea, Ehime, Japan. The samples collected were each filtered through a disposable syringe filter with a pore size of 0.45 μm (Sartorius Stedim Biotech GmbH, Minisart RC25), and stored in polypropylene bottles at 4°C.

2.2. Determination of sodium chloride

The electric conductivity of standard solutions of water, 1, 2, 3% NaCl solutions and real seawater samples were measured using a handheld conductivity meter (Eutech Instruments Pte. Ltd., CON 110). Approximate NaCl concentrations of real seawater samples were estimated from

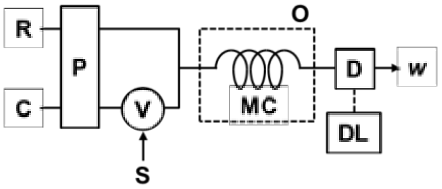

Fig. 1 Schematic of N-NO_2^- determination system by FIA. R, reagent; C, carrier; S, sample; P, peristaltic pump; V, 6-port valve; MC, mixing coil; O, oven; D, detector; DL, data logger; *w*, waste.

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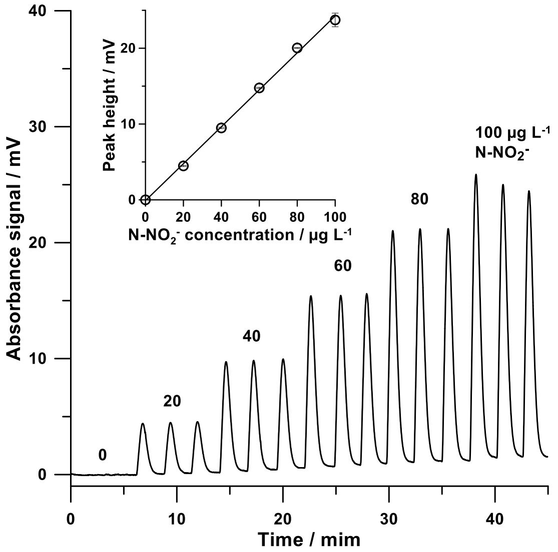

Fig. 2 Response signal to different concentration of $N\text{-}NO_2^-$ ranging from zero to 100 μg L^{-1}. The inset shows the calibration curve obtained from the $N\text{-}NO_2^-$ peak height. The error bars shown are one standard deviation, $n = 3$. The regression equation and determination coefficient of the calibration curve are $y = 0.2437x - 0.1060$ and $r^2 = 0.9979$, respectively.

their electric conductivities.

2.3. Determination of nitrite nitrogen

Nitrite nitrogen was determined by FIA based on the Griess method [6]. Figure 1 shows the schematic diagram for the nitrite determination. A reagent solution (R, 0.1% Sulfanilamide, 0.01% *N*-(1-Naphthyl)ethylenediamine dihydrochloride, 0.112 mol L^{-1} hydrochloric acid) and carrier solution (C, H_2O) were pumped by a peristaltic pump (P, Rabbit, Rainin Instrument Co. Inc.) at a flow rate of 0.40 mL min^{-1} each. A 200 μL of sample solution (S) was injected into the carrier flow by a 6-port auto valve (V, 401-03, FLOM, Inc.). The sample injected was merged with the reagent, and flowed into a Teflon mixing coil (MC, 0.96 mm i.d., 80 cm long) and an absorbance detector (D, SPD-10A, Shimadzu Corp.). The absorbance signal at 540 nm was recorded using a data logger (midi Logger GL200A, Graphtec Corp.). The mixing coil was placed inside an oven (O, 70°C, CS-300C, Chromato Science Co., LTD.) to keep the reaction temperature constant.

3. Results and Discussion

3.1. Analysis of nitrite nitrogen in pseudo-seawater using absolute calibration method

The performances of the $N\text{-}NO_2^-$ detection system was evaluated. Figure 2 shows the absorbance signal and the calibration curve in the $N\text{-}NO_2^-$ range of zero to100 μg L^{-1}. The relative standard deviations of repeated measurements for each $N\text{-}NO_2^-$ concentration (except zero) ranged from 0.26 to 3.80% ($n = 5$). The linearity of the calibration curve was satisfactory ($r^2 = 0.9979$), and the limit of detection ($S/N = 3$) was calculated to be 0.76 μg L^{-1} which meets the requirement to detect the standard value of $N\text{-}NO_2^-$ in Drinking Water Quality Standards in Japan (0.04 mg L^{-1} [5]). The throughput rate was 25 samples per hour.

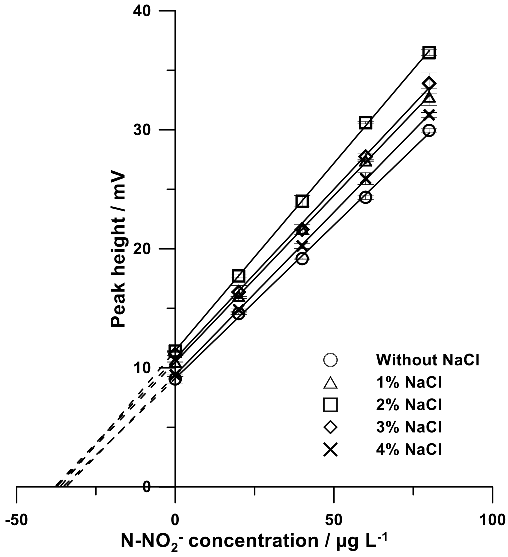

Fig. 3 Standard addition plots for pseudo-seawater samples. The error bars shown are one standard deviation, $n = 3$. The regression equation and determination coefficient for each pseudo-seawater sample are, respectively, $y = 0.2577x + 9.1033$, $r^2 = 0.9991$ for without NaCl; $y = 0.2798x + 10.473$, $r^2 = 0.9999$ for 1% NaCl; $y = 0.3151x + 11.434$, $r^2 = 0.9998$ for 2% NaCl; $y = 0.2844x + 10.782$, $r^2 = 0.9982$ for 3% NaCl; $y = 0.2734x + 9.4027$, $r^2 = 0.9999$ for 4% NaCl.

The $N\text{-}NO_2^-$ detection system was applied to the pseudo-seawater samples containing 40 μg L^{-1} $N\text{-}NO_2^-$ without and with 1, 2, 3, or 4% NaCl. The average $N\text{-}NO_2^-$ concentrations ($n = 3$) for the samples were calculated to be, respectively, 38.6 ± 0.4 μg L^{-1} for without NaCl, 44.0 ± 0.4 μg L^{-1} for 1% NaCl, 51.2 ± 0.1 μg L^{-1} for 2% NaCl, 49.6 ± 0.0 μg L^{-1} for 3% NaCl, 49.8 ± 0.1 μg L^{-1} for 4% NaCl. These results clearly demonstrate that the NaCl of percentage-order causes the significant positive error in $N\text{-}NO_2^-$ determination.

3.2. Analysis of nitrite nitrogen in pseudo-seawater using standard addition method

The pseudo-seawater samples were measured using the $N\text{-}NO_2^-$ detection system introducing standard addition method. The pseudo-seawater spiked 0, 20, 40, 60, or 80 μg L^{-1} of $N\text{-}NO_2^-$ samples were injected into the $N\text{-}NO_2^-$ detection system. The throughput rate was 5 samples per hour which was slower than the case where the absolute calibration method is used. Figure 3 shows the calibration curves for the pseudo-seawater samples containing 40 μg L^{-1} $N\text{-}NO_2^-$ without and with 1, 2, 3, or 4% NaCl. In all of these samples, the linearity of the calibration curve was satisfactory ($r^2 > 0.997$). The average $N\text{-}NO_2^-$ concentrations ($n = 3$) for the samples were calculated to be, respectively, 35.3 ± 1.4 μg L^{-1} for without NaCl, 37.4 ± 0.5 μg L^{-1} for 1% NaCl, 36.3 ± 0.7 μg L^{-1} for 2% NaCl, 37.9 ± 2.0 μg L^{-1} for 3% NaCl, 34.4 ± 0.4 μg L^{-1} for 4% NaCl. The relative standard deviation of the five $N\text{-}NO_2^-$ values above is only 4.6%.

3.3. Comparison of standard addition method with absolute calibration method

Table 1 Analytical results of real seawater samples, $n = 3$.

Sample	1	2	3	4
N-NO_2^- concentration using absolute calibration method / μg L^{-1}	22.0 ± 0.0$^{a)}$	37.4 ± 0.2$^{a)}$	32.2 ± 0.1$^{a)}$	19.0 ± 0.3$^{a)}$
N-NO_2^- concentration using standard addition method / μg L^{-1}	16.5 ± 1.6$^{b)}$	25.2 ± 1.8$^{b)}$	22.0 ± 1.8$^{b)}$	13.5 ± 2.9$^{b)}$
NaCl concentration, %	1.66	1.48	1.61	2.89

The samples 1 and 2 were collected from the mouth of the Imagire River, Tokushima, Japan. The samples 3 and 4 were collected from the coast of the Uwa Sea, Ehime, Japan. a) Average ± standard deviation of the N-NO_2^- concentration, b) average ± standard deviation of the x-intercept of the liner regression curve.

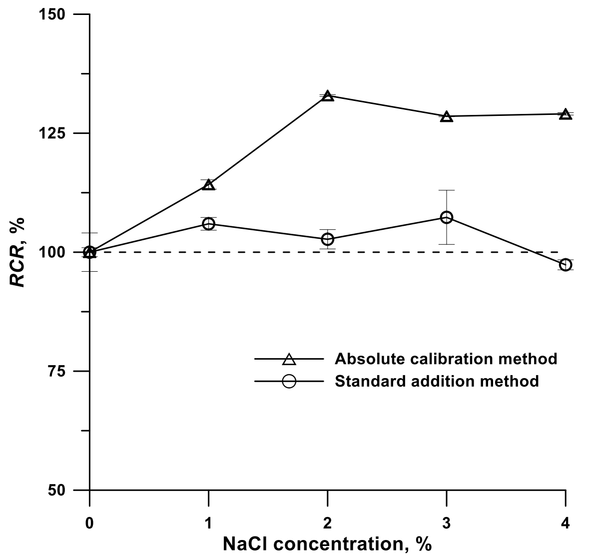

Fig. 4 Comparison of the *RCR*s obtained by standard addition method and absolute calibration method. The *RCR*s are calculated by Eq. (1). The error bars shown are one standard deviation, $n = 3$.

In order to easily compare the difference in N-NO_2^- concentrations with and without NaCl, the relative concentration ratio, *RCR*, was calculated by Eq. (1).

$$RCR = C_{\text{N-NO2-}} / C_{\text{N-NO2- wo NaCl}} \times 100\% \qquad (1)$$

where $C_{\text{N-NO2-}}$ is the N-NO_2^- concentration for the pseudo-seawater sample containing zero to 4% NaCl. $C_{\text{N-NO2- wo NaCl}}$ is the N-NO_2^- concentration for the sample without NaCl. The *RCR*s obtained by standard addition method and absolute calibration method are plotted on Fig. 4. The *RCR* of absolute calibration method is increased with the NaCl concentration. Approximately 30% of positive errors are found at 2, 3, and 4% NaCl pseudo-seawater samples. These errors might be caused by the promotion of the Griess reaction and/or the change in the refractive index of the solution due to the high degrees of NaCl, resulting in a decrease in the amount of light that reaches the photodetection element of absorbance detector. On the other hand, the *RCR* of standard addition method falls within the range of 97.4 to 107.3%. The use of standard addition method successfully suppresses the positive errors occurred in absolute calibration method.

3.4. Application to real seawater sample

The N-NO_2^- detection system was applied to the real seawater samples containing several percentages of NaCl. Table 1 summarizes the analytical results of real seawater samples. Note that the standard deviations of the N-NO_2^- concentration used in the two quantitative analysis approaches are differ as described in Table 1. In all of the seawater samples, the N-NO_2^- concentrations calculated by the absolute calibration method were clearly higher than the respective values by the standard addition method. This tendency was very similar to the analytical result of pseudo-seawater sample. It is likely, therefore, that the use of absolute calibration method leads to the overestimation of N-NO_2^- level in real seawater sample.

In summary, we have presented the FIA based on the Griess reaction for the determination of N-NO_2^-. The adoption of the standard addition method into the detection system enabled us to quantify the N-NO_2^- in the samples which contain relatively high degrees of salt. A disadvantage of the standard addition method is the extra time required for making the spiked samples and measurement. This issue would be improved dramatically by automating the sample preparation.

Acknowledgments

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