

Comparison of continuous flow analysis and ion chromatography for determinations of nitrate, nitrite and phosphate ions in seawater and development of related seawater certified reference materials

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Abstract

Determinations of three nutrients (nitrate, nitrite and phosphate ions) in seawater were investigated by continuous flow analysis (CFA) based on colorimetry and ion chromatography (IC). The accuracies of those methods were examined by discussing their uncertainties. While CFA is popular in this field, its comparison with IC was reported for the first time and contributed to an increase in the reliability of the analytical results. Finally, the investigation led to the development of three kinds of seawater certified reference materials (CRMs) of the National Metrology Institute of Japan (NMIJ) (NMIJ CRM 7601-a, 7602-a and 7603-a) for which the property values of nutrients including dissolved silica were given. The details of the development are described in the present paper.

Introduction

Nitrate, nitrite and phosphate ions as well as dissolved silica, which are so-called nutrients, are important to monitor the variation of the ecosystem in seawater [1, 2]. For the studies of global warming and ocean general circulation, the concentrations of the nutrients in seawater are measured all over the world; for example, in global projects such as World Ocean Circulation Experiment [1]. For such measurements, it is a main problem to accurately monitor spatial and temporal variations of the nutrients concentrations; however, it is not easy because suitable concentration scales are exiguous [3]. Under such circumstances, it has been needed to develop reliable certified reference materials (CRMs) and consequently to obtain both the comparability of the measurement and the traceability to the International System of Units (SI) [4].

A few years ago, existing SI traceable CRM for nutrients in seawater was only MOOS-2 produced by the National Research Council (NRC) Canada [5], followed by the subsequent CRM MOOS-3 with smaller uncertainties has been released [6]; however, both of CRMs were based on the seawater in the North Atlantic Ocean with middle nutrient level. The oceanography community required CRM not only whose certified value accompanied a small uncertainty but whose nutrient level was as high as that of the Pacific Ocean. To meet the demand for such CRMs, the National Metrology Institute of Japan (NMIJ) has developed three kinds of seawater CRMs for a nutrient analysis whose certified values are SI traceable.

To determine the certified values of CRMs, more than one kind of measurement method is usually required; therefore, we used two methods to measure the nutrients. One is a traditional colorimetry by continuous flow analysis (CFA) [1, 7–9] and the other is ion chromatography (IC) based on separation of ion exchange and detection by electric conductivity or UV-visible absorbance, which is often used for measuring inorganic anions and can separate target ions from a sample solution efficiently [10, 11]. In the present paper, a comparison of the two methods, which is reported for the first time, and the CRM certification process are discussed on the three nutrient ions.

Seawater matrix often affects measurements of chemical components in seawater. To take account of the problem, a standard addition method is usually used for the measurement of the samples containing

a high concentration matrix-like seawater. Although IC can normally separate the matrix components from the analyte, the standard addition method was adopted because it has the advantage of eliminating the density difference between measured samples and standard solutions. The availability of desalination using off-line columns and the difference of analytical performance due to analytical columns were also examined to confirm the effect of seawater matrix on the IC measurements. While the previous study has shown that the standard addition method is unsuitable for CFA due to the narrow linear range of its calibration curve [12]. Therefore, for CFA, a bracketing method using two standard solutions matched matrices with artificial seawater (ASW) was examined. The applicability of each analytical method to the nutrients in seawater was validated by recovery tests using ASW samples with known concentrations of nutrients. In the present paper, the first comprehensive examination on IC methods for the nutrients in seawater is reported.

The mass fraction of each analyte in three kinds of candidate NMIJ CRMs was measured with the two analytical methods, and each property value was decided by combining those results. The magnitudes of the uncertainties for the nutrient mass fraction in the developed CRMs have fulfilled minimum requirements in the oceanography community. Moreover, one of them is the first CRM that can be utilized for measurements of high nutrient concentrations in the Pacific Ocean. The CRMs with property values that have such small uncertainties will contribute to advancing studies on seawater nutrients in oceanography.

Experimental

Apparatus for CFA

A continuous flow analyzer AACS-V (BLTEC, Osaka, Japan) which automatically carried out a colorimetry was employed for measurements of the nutrients. The sample solutions were injected with an auto-sampler, in which a sample aliquot of 1.8 mL was stored in each of the vials covered by polytetrafluoroethylene (PTFE) septa. A reduction column (Glastron, Inc., NJ, USA) was used to convert nitrate to nitrite, which was made of coiled hollow cylindrical cadmium. Three reduction columns were incorporated in the CFA reaction line; the columns had been treated before use by a copper sulfate solution. The complete conversion was allowed through the three columns [13]. Flasks, beakers and spoons used for the preparation of the reagents and measuring solutions, which are the solutions just before being mixed with the reagents in the analyzer, were all made from any polymethylpentene, polyethylene or PTFE. Storage bottles for the reagents and the measuring solutions were made from polypropylene.

Apparatus For Ic

The IC system in the present study consisted of a gradient pump GP-40 (Dionex, Sunnyvale, USA), a column oven LC-30 (Dionex), an autosampler AS (Dionex) with a 50 μ L sample loop, a self-regenerating

suppressor SRS-300 (Dionex), a UV-visible detector AD-20 (Dionex), and an electric conductivity detector CD-20 (Dionex). Separation was achieved with each of the following three anion-exchange columns (Dionex): (1) an IonPac AS10 analytical column (250 mm x 4 mm) with an IonPac AG10 guard one (50 mm x 4 mm), (2) an IonPac AS12A analytical column (200 mm x 4 mm) with an IonPac AG12A guard one (50 mm x 4 mm), and (3) an IonPac AS23 analytical column (250 mm x 4 mm) with an IonPac AG23 guard one (50 mm x 4 mm). The flow rates were 1.0 mL min^{-1} for both AS10 column and AS23 one, and 1.5 mL min^{-1} for AS12A one. Ahead of some IC measurements, desalination for chloride, bromide, iodide and sulfate ions from seawater sample was achieved with the following cartridges (Dionex): OnGuard II Ag (2.5 mL), OnGuard II Ba (2.5 mL) and OnGuard II Na (2.5 mL). The last cartridge was used to remove silver and barium ions leaked from the former two cartridges.

Seawater Samples

Three kinds of seawater samples were used for measurements. The seawater samples with an extremely low concentration of nutrients (ELSW) and with a high concentration of nutrients (HSW) were collected from the surface layer in the Pacific Ocean and from the nutrient maximum layer in the Pacific Ocean, respectively. The seawater sample with a middle concentration of nutrients (MSW) was prepared by blending the following three seawaters: (1) 690 m depth seawater of the Arctic Sea collected with cooperation of JAMSTEC, (2) 1500 m depth one of the Atlantic Ocean collected with the cooperation of Meteorological Research Institute, Japan and the Plymouth Marine Laboratory, UK, and (3) 397 m depth one of the Suruga-wan Bay, to approximate the nutrient concentration in the North Atlantic Ocean. The nutrient composition of the second one was slightly changed during transportation, but that of the blended seawater is similar to the original composition of the 1500 m depth seawater of the Atlantic Ocean. Detail descriptions of the seawater samples are provided elsewhere [14].

To validate the IC method, the existing seawater CRM (MOOS-2) developed by NRC Canada was used.

Reagents

All reagents were of analytical reagent grade available from FUJIFILM Wako Pure Chemical Co. Inc., Tokyo, Japan, unless otherwise specified. Water was purified by a Milli-Q Integral Q-POD Element system (Merck Millipore, Darmstadt, Germany).

NMIJ CRMs 3805-a, 3806-a and 3808-a supplied by NMIJ were used as the standard solutions of nitrate, nitrite and phosphate ions, respectively.

ASW, which was used as CFA carrier solution and to prepare samples for the recovery test, was prepared according to the literature[1].

Preparation Of Reagent Solutions For Cfa

All the coloring reagent solutions were prepared as described in the literature [15]. The Griess method [16–18] was employed for the measurement of nitrite and nitrate; nitrate was measured after being reduced to nitrite. For the Griess method, the following three solutions were prepared: (1) a 0.09 mol L⁻¹ imidazole solution as a catalyst containing both 0.02 mol L⁻¹ sulfuric acid and 0.1% TritonX-100, (2) a 0.06 mol L⁻¹ sulfanilamide solution as a modifier containing both 1.2 mol L⁻¹ hydrochloric acid and 0.1% TritonX-100, and (3) a 0.004 mol L⁻¹ N-1-naphthylethylenediamine dihydrochloride (1-NED) solution as a coupling agent containing 0.12 mol L⁻¹ hydrochloric acid.

For measurement of phosphate, the phosphomolybdenum blue method [19, 20] was employed. First, a 0.02 mol L⁻¹ stock molybdate solution was prepared by dissolving 5.6 g of disodium molybdate dihydrate and 0.12 g of antimonyl potassium tartrate in *ca.* 500 mL water containing 35 mL of sulfuric acid, diluting to 1000 mL by water, and finally adding 5 mL of a 15% solution of sodium dodecyl sulfate. Finally, the following two solutions were prepared: (1) a solution as a mixed coloring agent, prepared by dissolving 1.1 g of L-ascorbic acid in 200 mL of the 0.02 mol L⁻¹ stock molybdate solution, and then mixing 4 mL of a 15% solution of sodium dodecyl sulfate, and (2) 0.04 mol L⁻¹ sulfuric acid as a pH controlling agent containing 0.075% sodium dodecyl sulfate.

Preparation Of Measuring Solutions For Cfa

For measurements using CFA, the bracketing method [12] was applied to those seawater samples, and ten bottles of each seawater sample (a candidate NMIJ CRM) were usually analyzed in parallel. For each measurement, seawater samples were diluted with water to the following seawater (or ASW) containing rate: 0.04 g g⁻¹ for measurements of nitrate in HSW, 0.50 g g⁻¹ for nitrate in MSW and 0.75 g g⁻¹ for nitrate in ELSW, nitrite and phosphate in HSW, MSW and ELSW. To calibrate the mass fraction of the nutrients, two standard solutions which had 5% lower and 5% higher mass fractions than the diluted seawater samples, whose matrixes were matched with those samples using ASW, were also prepared. All preparations and measurements were carried out in a laboratory at a temperature between 24 °C and 26 °C throughout the present study.

Measurement Procedures For Cfa

The flow diagram of CFA and the flow rate are shown in Fig. 1 and its supporting explanation is in Table 1. All reactions and detection were performed on a single continuous flow. The detailed description of operating the CFA instrument and a calculation way of the bracketing method is the same as the previous report [12]. The absorption of the complex formed by the reaction was detected at 550 nm for nitrite and nitrate ions and at 80 nm for phosphate ions. In the measurement of nitrate ions, the absorption was provided as the sum of “nitrite and nitrate ions” because nitrate ions were reduced to nitrite ions as mentioned above. The mass fraction of nitrate ions in a sample was calculated by

subtracting the amount-of-substance content ($\mu\text{mol kg}^{-1}$) of nitrite ions from the total content of “nitrite and nitrate ions” and converting it into a mass fraction.

Table 1
Detail of each item in the flow diagram

	For Nitrite	For Nitrite + Nitrate	For Phosphate
Reagent A ^{*1}	Sulfanilamide ^{*3}	Imidazole ^{*3}	Mixed coloring agent ^{*3}
Reagent B ^{*1}	1-NED ^{*3}	Sulfanilamide ^{*3}	Sulfuric acid ^{*3}
Reagent C ^{*1}	-	1-NED ^{*3}	-
Reaction coil D ^{*1}	5 turns	5 turns	20 turns
Reaction coil E ^{*1}	5 turns	5 turns	55 turns
Reaction coil F ^{*1}	15 turns	15 turns	-
Reduction columns ^{*2}	None	Inserted	None
Wavelength of colorimeter	550 nm	550 nm	880 nm
*1 The symbols (A to F) correspond to those in Fig. 1. *2 See Fig. 1. *3 Regarding each exact composition, see in the text.			

Preparation Of Eluents For Ic

The eluents for IC were prepared by dissolving hydrochloric acid, sodium carbonate, sodium hydrogen carbonate or sodium chloride (Wako) in water: 80 mmol L⁻¹ HCl for AS10 column in measuring nitrate ions, 80 mmol L⁻¹ NaCl for the same column in measuring nitrite ions, 2.7 mmol L⁻¹ Na₂CO₃ containing 0.3 mmol L⁻¹ NaHCO₃ for AS12A one, and 4.5 mmol L⁻¹ Na₂CO₃ containing 0.8 mmol L⁻¹ NaHCO₃ for AS23 one.

Desalination Procedure For Ic

Sample solutions for the standard addition method were prepared by adding 0 mg kg⁻¹ (none), 2 mg kg⁻¹, 4 mg kg⁻¹ and 6 mg kg⁻¹ nitrate ions and individually diluting to 0.95 g g⁻¹ seawater by water. Each sample solution was desalinated for chloride, bromide and iodide ions by passing through both an OnGuard II Ag cartridge and an OnGuard II Na one. The OnGuard II Na cartridge was used for removing silver ions excessively released from the OnGuard II Ag cartridge. In the case of desalination for chloride,

bromide, iodide and sulfate ions, an OnGuard II Ba cartridge was further used in combination; the filtrates were directly analyzed by IC under the condition mentioned above with no further treatment.

Results And Discussion

Analytical results of nutrient ions by CFA

For nitrate, nitrite and phosphate ions, five recovery tests were carried out as follows: the seawater samples were diluted to the respective content specified in "Preparation of measuring solutions for CFA" and known amount of the nutrient ion standard solutions were added. The measuring solutions before and after addition were measured by the CFA bracketing method. The differences, the experimental addition mass fractions, were compared to the addition mass fractions in the gravimetric preparation. The results are shown in Table 2. For nitrate ions, except for the analysis of the lowest addition mass fraction, it was confirmed that for each artificial sample nearly 100% nitrate ions were recovered almost within the expanded uncertainty with a coverage factor of 2, $U(k=2)$, derived from the uncertainty of the bracketing method including the repeatability. Although the tests for nitrate ion of the lowest mass fraction, nitrite and phosphate ions showed deviations beyond the repeatability due to the low absolute mass fractions to be measured, it was determined that they were acceptable considering the uncertainty required for oceanographic observations. They were included in the uncertainty of the analytical results as matrix difference between sample and standard solutions, as shown in the previous report [12].

Table 2
Results of recovery tests for nutrient ions by CFA method.

Nutrient	Base Seawater	Seawater containing rate	Added	Found ^{*4}	Recovery ^{*4}
			w_{pre} / $mg\ kg^{-1}$ 1	w_{det} / $mg\ kg^{-1}$	w_{det} / w_{pre}
NO ₃ ⁻	HSW	0.04 ^{*1}	0.1046	0.1046 ± 0.0004	100.0% ± 0.4%
	MSW	0.50 ^{*2}	0.0459	0.0458 ± 0.0002	99.9% ± 0.5%
	ELSW	0.75 ^{*3}	0.00010	0.00008 ± 0.00001	75.2% ± 16.8%
NO ₂ ⁻	MSW	0.75	0.00159	0.00164 ± 0.00002	102.7% ± 1.3%
PO ₄ ³⁻	HSW	0.75	0.0208	0.0202 ± 0.0002	97.3% ± 0.8%

^{*1} Corresponding to HSW analysis. ^{*2} Corresponding to MSW analysis. ^{*3} Corresponding to ELSW analysis. ^{*4} Each figure following ± indicates the expanded uncertainty due to bracketing method including the repeatability ($k=2$).

Based on the discussion, three kinds of seawater samples (candidate NMIJ CRMs) were analyzed by CFA, whose results were shown in Table 3.

Table 3
Measured values of each candidate NMIJ CRM by CFA method ^{*1}

	Mass fraction for HSW / mg kg ⁻¹	Mass fraction for MSW / mg kg ⁻¹	Mass fraction for ELSW / mg kg ⁻¹
NO ₂ ⁻	0.00123 ± 0.00013 (n = 2)	0.0183 ± 0.0007 (n = 2)	0.00165 ± 0.00012 (n = 2)
NO ₃ ⁻	2.7528 ± 0.0071 (n = 4)	0.9565 ± 0.0020 (n = 4)	0.0013 ± 0.0003 (n = 2)
PO ₄ ³⁻	0.2876 ± 0.0010 (n = 2)	0.1013 ± 0.0010 (n = 2)	0.0015 ± 0.0002 (n = 2)

^{*1} Each figure following ± indicates the combined standard uncertainty. The combined standard

uncertainty u_c was calculated from the following equation $u_c = \sqrt{(s_r/\sqrt{n})^2 + u_{\text{mtrx}}^2 + u_{\text{std}}^2}$,

where n is a number of replication of measurements using ten bottles, s_r is repeatability SD obtained by analysis of variance from measured values for ten bottles $\times n$, u_{mtrx} is the uncertainty due to matrix difference between the sample and standard solution sets (refer to the previous report [12]) and u_{std} is the standard uncertainty due to the standard solution of the analyte.

Analytical Results Of Nitrate Ions By Ic

The linearity of each calibration curve for nitrate ions in both UV absorbance detection and electric conductivity detection was verified since the coefficient of correlation of the calibration curve for each detection was more than 0.9999 in the mass fraction range between 0.8 mg kg⁻¹ and 8.3 mg kg⁻¹ with dilution to 0.95 g g⁻¹ seawater. Therefore, the mass fractions of nitrate ions in both HSW and MSW were measured by the standard addition method.

In the case of the AS10 column with an HCl eluent, the mass fractions of nitrate ions in both HSW and MSW were precisely measured with UV absorbance detection (Fig. 2), although they could not be measured with electric conductivity detection because of a huge vicinity peak of chloride ions and an unstable baseline. Although nitrate ions could be detected more sensitively at the wavelength of 210 nm, the wavelength of 225 nm was chosen from the viewpoint of avoiding interference of bromide ions because the molar absorption coefficient of bromide ions in longer wavelength became rapidly lower than that of nitrate ions [21]. On the other hand, when bromide ions were efficiently removed by the desalination procedure for chloride, bromide and iodide ions, the more effective wavelength of 210 nm could be used with no interference of bromide ions. Although the detection at 210 nm with the desalination was advantageous in the sensitivity of nitrate ions compared to the detection at 225 nm with no desalination, the uncertainty of the quantitative result was not improved because of the addition

of tedious procedure. It should be noted that the quantitative value of the detection at 225 nm with no desalination agreed with that of the detection at 210 nm with desalination (Table 4). This fact partly supported no interference from any coexisting ions.

Table 4
Measured mass fractions of nitrate ions in candidate NMIJ CRMs by IC method

	Pretreatment *1	Column / Eluent	Detection	HSW *2	MSW *2	ELSW *2
A	only dilution	AS10 / HCl	UV absorbance (225 nm)	2.748 ± 0.010 (n = 5)	0.934 ± 0.005 (n = 4)	DL = 0.005 *3
B	only dilution	AS23 / carbonate	UV absorbance (210 nm)	2.748 ± 0.021 (n = 1) *5	0.937 ± 0.009 (n = 1) *6	-
C	only dilution	AS23 / carbonate	electric conductivity	2.725 ± 0.031 (n = 1) *5, *7	0.936 ± 0.015 (n = 1) *6, *7	-
D	desalination- 1	AS10 / HCl	UV absorbance (210 nm)	2.751 ± 0.026 (n = 2)	0.936 ± 0.028 (n = 1)	-
E	desalination- 1	AS12A / carbonate	UV absorbance (210 nm)	2.724 ± 0.032 (n = 2)	0.932 ± 0.022 (n = 1)	-
F	desalination- 1	AS12A / carbonate	electric conductivity	2.759 ± 0.024 (n = 1)	0.931 ± 0.012 (n = 1)	-
G	desalination- 2	AS12A / carbonate	UV absorbance (210 nm)	2.738 ± 0.026 (n = 1) *4, *7	-	-
H	desalination- 2	AS12A / carbonate	electric conductivity	2.701 ± 0.019 (n = 1) *4	-	-

*1 Desalination-1 means desalination for Cl⁻, Br⁻ and I⁻ by the OnGuard II Ag cartridge. Desalination-2 means desalination for Cl⁻, Br⁻, I⁻ and SO₄²⁻ by both the OnGuard II Ag cartridge and the OnGuard II Ba one.

*2 Units of each value are mg kg⁻¹. The *n* value in each parenthesis means the number of the replication for independent measurements by the standard addition method. Each value after "±" means the combined standard uncertainty estimated from both the calibration curve of the standard addition method and the concentration uncertainty of the standard solution. The combined standard uncertainty

$$u_c = \frac{1}{n} \sqrt{\sum_{i=1}^n u_i^2}$$

u_c was calculated from the individual standard uncertainties u_i using the following equation

*3 DL indicates the detection limit. *4 *5 *6 Both detections with UV absorbance and electric conductivity were simultaneously carried out in every single run. *7 The results were not utilized to determine the certified values of the NMIJ CRMs.

The recovery test for the IC method was also carried out at 225 nm without desalination using the artificial sample. The artificial sample was prepared by adding nitrate of 2.908 mg kg^{-1} (equivalent to HSW) to ELSW, whose nitrate mass fraction was up to 0.001 mg kg^{-1} . The measured concentration, 2.919 mg kg^{-1} , agreed with the mass fraction in the gravimetric preparation within the expanded uncertainty with a coverage factor of 2 ($U(k=2)$), 0.018 mg kg^{-1} , derived from the calibration curve of the standard addition method. The IC method is operationally simple and reliable, although it is less sensitive than CFA. On the other hand, CFA is highly sensitive, but its operation is much complicated.

To further validate, the existing CRM, MOOS-2, distributed from NRC Canada was analyzed under the condition of the detection at 225 nm with no desalination by using the AS10 column. The certified value of nitrate ions was converted from the amount-of-substance content (nitrite, and “nitrite and nitrate”) in the unit of $\mu\text{mol L}^{-1}$ to mass fractions in that of mg kg^{-1} using the measured density of 1.0230 g L^{-1} (with a relative standard deviation of five measurements, 0.01 %) at 25.0°C . As a result, the measured value of nitrate ions in MOOS-2 ($1.335 \pm 0.026 \text{ mg kg}^{-1}$), agreed with the certified value ($1.339 \pm 0.063 \text{ mg kg}^{-1}$). The accuracy of the present method was verified at least within the $U(k=2)$ of MOOS-2.

In case that the seawater samples were desalinated for chloride, bromide and iodide ions, nitrate ions could be determined by using an anion-exchange column with low capacities such as an AS12A column under the conditions of both electric conductivity detection and UV absorbance detection at 210 nm. The measured values from both detection ways agreed with each other (Table 4). Figure 3 shows chromatograms of HSW by using the AS12A column after the desalination with only an OnGuard II Ag cartridge. A large peak of sulfate ions disappeared when the seawater sample was desalinated for sulfate as well as chloride, bromide, and iodide ions; the measured values of nitrate ions were substantially identical for both electric conductivity detection and UV absorbance detection at 210 nm. There was also no significant difference between the measured values in the two desalination modes.

In case that the high-capacity anion-exchange column (IonPac AS23) was used with a carbonate eluent, nitrate ions could be determined when the seawater sample was diluted to 29% by water (Fig. 4). The measured values of nitrate ions (Table 4) well agreed with those under the other measurement conditions mentioned above. Although Ohguni *et al.* reported direct determination of nitrate in seawater by IC using a high-capacity anion-exchange column [22], the accuracy of their results was at several percent levels even for the relatively high mass fraction of nitrate.

As described above, regardless of the different measurement conditions such as pretreatments of the seawater sample (only dilution or two modes of the desalination), separation conditions (columns and eluents) and detection ways (electric conductivity or UV absorbance), the measured values of nitrate ions agreed with each other within their expanded uncertainties estimated for the standard addition method (Table 4). This fact strongly suggests that any coexisting ions in the seawater samples did not interfere with the measured values for nitrate ions.

Analytical Results Of Nitrite And Phosphate Ions By Ic

Nitrite ions in the seawater samples were measured by using the AS10 column with an eluent of 80 mmol L⁻¹ NaCl and detecting UV absorbance at 210 nm (Fig. 5) [21]. Because recovery of nitrite ions with the desalination was a little varied, an analysis with no desalination was only carried out. The mass fraction of nitrite ions was (0.0202 ± 0.0030) mg kg⁻¹ for MSW (in this section, each value following "±" indicates the $U(k=2)$). The nitrite mass fractions for both HSW and ELSW were less than the detection limit (DL) (0.009 mg kg⁻¹ in an undiluted sample), which was defined as a mass fraction corresponding to three times the standard deviation for baseline noise near the retention time of nitrite ions. The reason for diluting the seawater samples despite its low mass fractions was that a negative peak appeared around the retention time of 5.5 min with no sample dilution and the peak interfered in the baselines.

Phosphate ions in the seawater samples were measured in the same way as nitrate ions using the AS23 column (Fig. 4). The desalination was not applied for the same reason as nitrite ions using the AS10 column. The mass fractions of phosphate ions in the seawater samples by IC were (0.243 ± 0.030) mg kg⁻¹, (0.109 ± 0.054) mg kg⁻¹, and (0.019 ± 0.016) mg kg⁻¹ for HSW, MSW, and ELSW, respectively. The mass fraction of phosphate ions in ELSW was close to the DL of this method.

Homogeneity Of The Candidate Nmij Crms

According to ISO guide 35 [23], the homogeneities of the seawater samples (candidate NMIJ CRMs) were evaluated based on the analytical results of 10 bottles × 2 times (4 times for nitrate in MSW, HSW and dissolved silica in 3 levels of seawater) by CFA. All the uncertainty due to the homogeneity was shown in Table 5 with that for dissolved silica. Although the homogeneity of the ELSW and a part of HSW are poorer than others due to the extremely low mass fractions of the analyte in those, these amount-of-substance components were conclusively decided as indicative values or information as described later section.

Table 5
Standard uncertainty due to the homogeneity for the candidate NMIJ CRMs.

	HSW / mg kg ⁻¹	MSW / mg kg ⁻¹	ELSW / mg kg ⁻¹
NO ₂ ⁻	0.00020 (15.9%)	0.00007 (0.36%)	0.00017 (10.3%)
NO ₃ ⁻	0.0082 (0.30%)	0.0007 (0.07%)	0.0001 (6.2%)
PO ₄ ³⁻	0.0007 (0.23%)	0.0002 (0.22%)	0.0007 (42%)
Dissolved silica (as Si)	0.0017 (0.04%)	0.0005 (0.06%)	0.00005 (0.15%)
* Each value in parentheses indicates the relative value to the property value.			

Stability Of The Candidate NmiJ Crms

The long-term stability of the seawater samples (candidate NMIJ CRMs) was estimated based on the analytical results up to almost 1500 days by CFA. According to ISO guide 35 [23], the trend analysis was performed for the variation of each nutrient mass fraction against the elapsed time, except the components were finally decided as information. As a result, no instability was found for any certified components. The standard uncertainty due to the long-term stability was estimated basically by multiplying the standard deviation of the slope by the time to the expiration date. All the uncertainty due to the long-term stability was shown in Table 6 with that for dissolved silica.

Table 6
Standard uncertainty due to the long-term stability for the candidate NMIJ CRMs.*

	HSW / mg kg ⁻¹	MSW / mg kg ⁻¹	ELSW / mg kg ⁻¹
NO ₂ ⁻	0.0002 (14%)	0.0002 (0.90%)	0.00023 (14%)
NO ₃ ⁻	0.0221 (0.81%)	0.0098 (1.0%)	0.00044 (32%)
PO ₄ ³⁻	0.0022 (0.75%)	0.0021 (2.1%)	0.00052 (34%)
Dissolved silica (as Si)	0.0078 (0.19%)	0.0045 (0.53%)	0.00052 (1.5%)
* Each value in parentheses indicates the relative value to the property value.			

Comparison of the results by CFA and IC and determination of certified values of the candidate NMIJ CRMs

All analytical results of the candidate NMIJ CRMs were compared. For nitrate ions, all the results listed in Tables 3 and 4 were used. The results for HSW were in good agreement with each other. For MSW, all the results were also in general agreement. For ELSW, although the result by IC was below the DL (0.005 mg kg^{-1} as mentioned above), the result by CFA was consistent with the estimation by the DL for IC. The results show CFA and IC can give comparable analysis for those seawater nutrients although sensitivity and the DL level of the analytical methods were different. Although seawater matrix might cause a slight difference between CFA and IC in the case of a low nutrient concentration, that was estimated as uncertainty due to difference among analytical techniques (mentioned below).

The certified values were calculated as an arithmetic means of the quantitative values measured by following three analytical techniques: CFA, IC direct measurement (without desalination) and IC with desalination. The quantitative values by IC with/without desalination were determined as follows, respectively: in case that two simultaneous detections by UV absorbance and electric conductivity were utilized for nitrate (BC, EF and GH in Table 4), a single measured value with a smaller uncertainty was chosen from both detection ways. Then, weighted means were taken for the direct measurement and the measurement with desalination, respectively, using the uncertainty due to the measurements as weight. For evaluating the uncertainty of the certified values, the differences among the analytical techniques, the homogeneity and the stability were taken into consideration. In the case of ELSW, a reliable certified value could not be estimated because of the low mass fraction; then, a property value was not given as a certified value, but just as information of a quantitative value using CFA.

On the nitrite analysis, comparison among the analytical methods could be demonstrated only for MSW due to very low mass fractions of nitrite ions. As a result, the quantitative values by CFA and IC without desalination were in good agreement with each other. A certified value was given to only MSW as an arithmetic means of both results. For nitrite ions in HSW and ELSW, property values were given as information the same as nitrate in ELSW. Due to the relatively low mass fractions of phosphate ions, those uncertainties were extremely high for IC; therefore, the property values of phosphate ions were decided as indicative ones for HSW and MSW, and as information for ELSW, only from the results by CFA.

The certified values, the indicative ones and the information are summarized in Table 7. The certified values for dissolved silica are also shown together in Table 7.

Table 7
 Certified values of the three NMIJ CRMs (HSW, MSW and ELSW)^{*1}

	HSW / mg kg ⁻¹	MSW / mg kg ⁻¹	ELSW / mg kg ⁻¹
NO ₂ ⁻	0.0012 ^{*3}	0.019 ± 0.002	0.0016 ^{*3}
NO ₃ ⁻	2.745 ± 0.050	0.942 ± 0.027	0.0013 ^{*3}
PO ₄ ³⁻	0.288 ± 0.005 ^{*2}	0.101 ± 0.005 ^{*2}	0.002 ^{*3}
Dissolved silica (as Si)	4.106 ± 0.043	0.837 ± 0.018	0.036 ± 0.004
^{*1} Mass fraction (mg kg ⁻¹). Each value after "±" indicates the expanded uncertainty $U(k=2)$.			
^{*2} Indicative value.			
^{*3} Provided as information, that means a quantitative value in time using only one analytical method. Since final uncertainty was large and the reliability as a property value was not sufficient, these values were provided as that.			

Conclusions

In the process of developing NMIJ CRMs for analysis of the nutrient ions in seawater, accuracies of the analyses by CFA based on colorimetry and IC were investigated. Both methods were validated by recovery tests. For IC, a standard addition method was examined for IC in the present study because it has the advantage of eliminating the density difference among measuring solutions. For CFA, the bracketing method with matrix matching by ASW was examined instead of the standard addition method in terms of the linearity of the calibration curve. As the result, it was ascertained that the bracketing method and the standard addition method applied to the seawater nutrient analyses by CFA and IC, respectively. Using these IC and CFA methods, three kinds of NMIJ CRMs, whose nutrients' concentrations range from zero concentrations to high concentrations equivalent to the nutrient maximum layer in the Pacific Ocean, were supplied worldwide. The NMIJ CRMs contribute to improving the accuracy of oceanographic observations.

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Figures

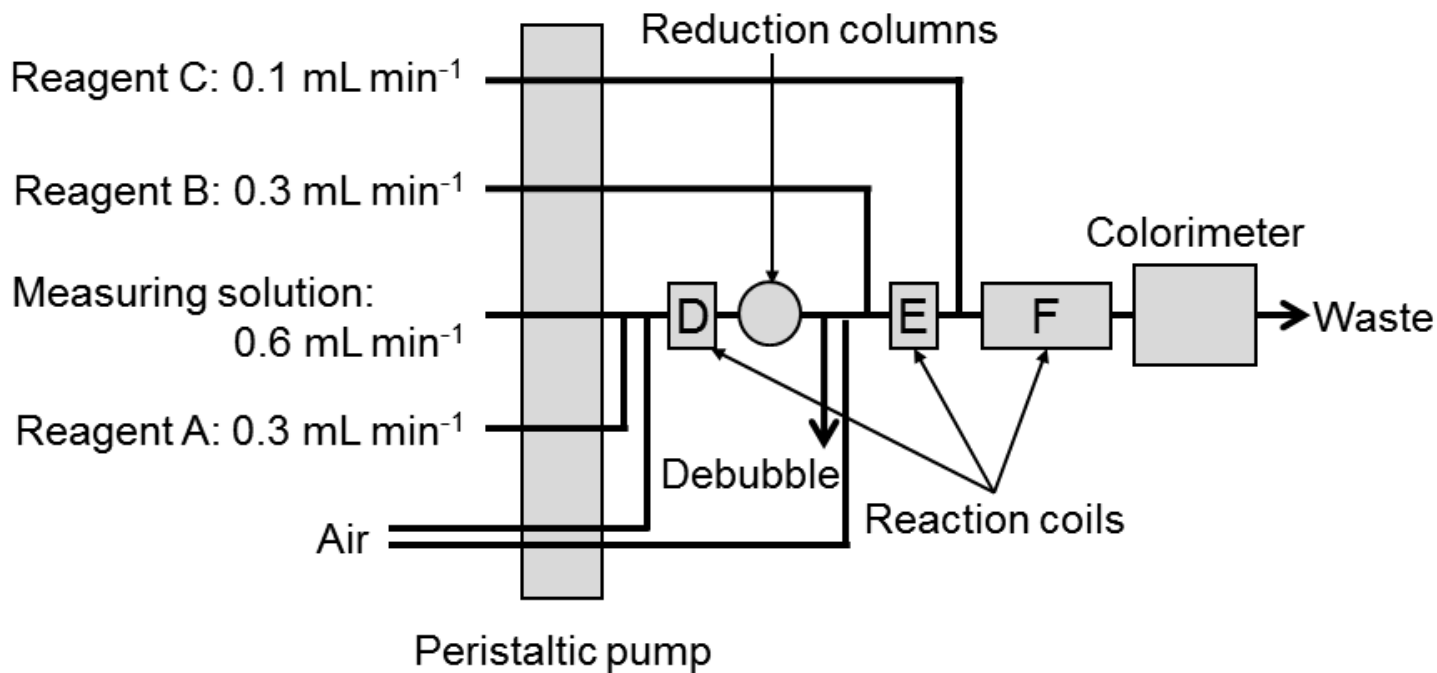


Figure 1

Flow diagram for CFA.

See the explanation in Table 1

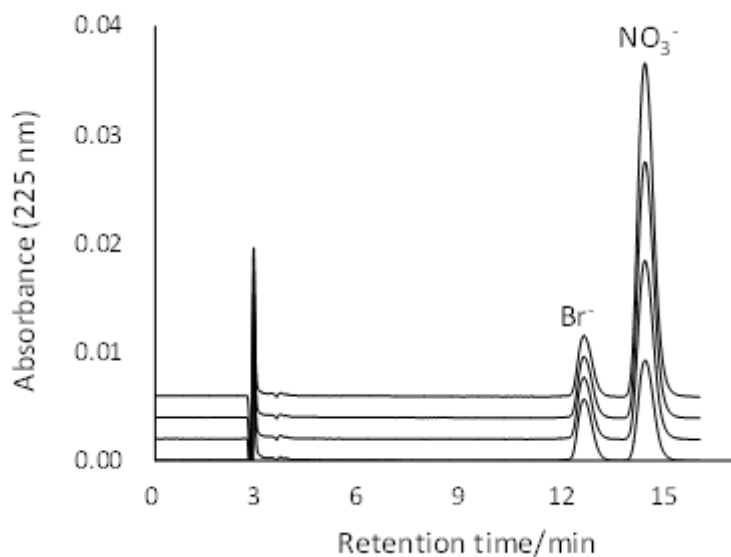


Figure 2

Chromatograms of seawater sample (HSW).

Pretreatment: only dilution to 0.91 g g⁻¹; column: AS10; eluent: 80 mmol L⁻¹ HCl; wavelength: 225 nm.

Chromatogram: (bottom) no addition of standard solutions; 6.0 mg kg⁻¹ nitrate added for the largest. In

Figs. 3 to 6, some offset is given to each baseline to avoid the chromatograms overlapping

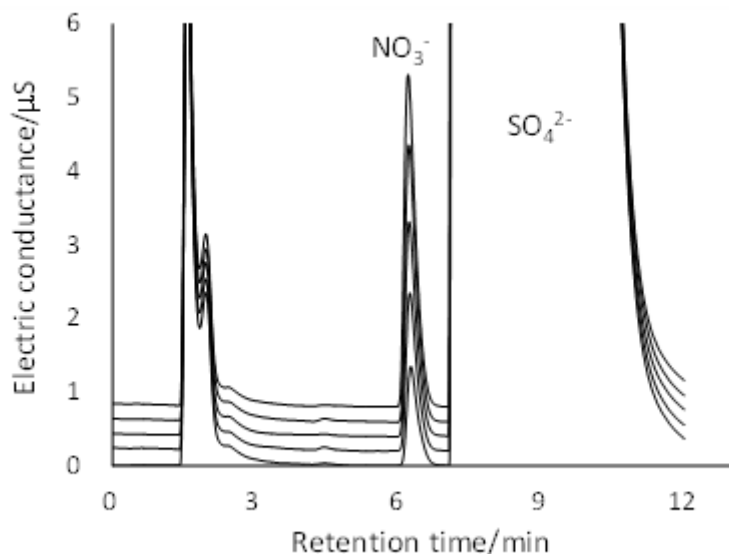


Figure 3

Chromatograms of seawater sample (HSW).

Pretreatment: desalination for chloride, bromide and iodide with dilution to 0.97 g g^{-1} ; column: AS12A; eluent: $2.7 \text{ mmol L}^{-1} \text{ Na}_2\text{CO}_3 + 0.3 \text{ mmol L}^{-1} \text{ NaHCO}_3$. Chromatogram: (bottom) no addition of standard solutions; addition of 6.0 mg kg^{-1} nitrate for the largest

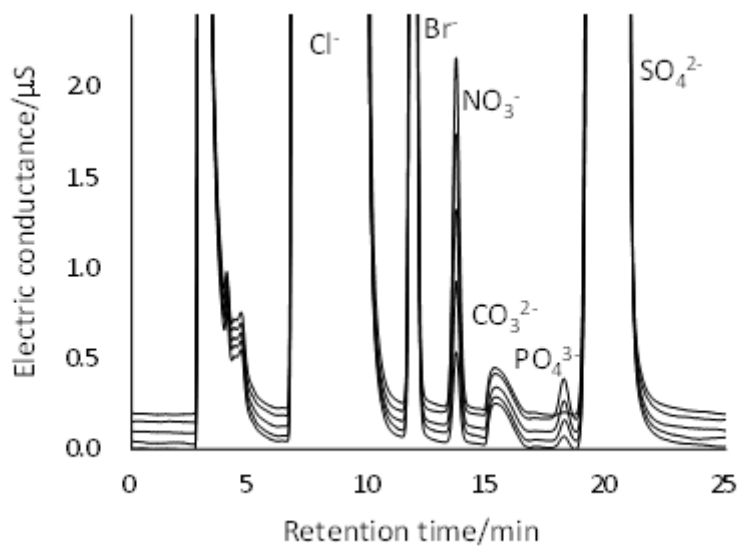


Figure 4

Chromatograms of seawater sample (HSW).

Pretreatment: only dilution to 0.29 g g^{-1} ; column: AS23; eluent: $4.5 \text{ mmol L}^{-1} \text{ Na}_2\text{CO}_3 + 0.8 \text{ mmol L}^{-1} \text{ NaHCO}_3$. Chromatogram: (bottom) no addition of standard solutions; addition of 2.3 mg kg^{-1} nitrate and 1.0 mg kg^{-1} phosphate for the largest

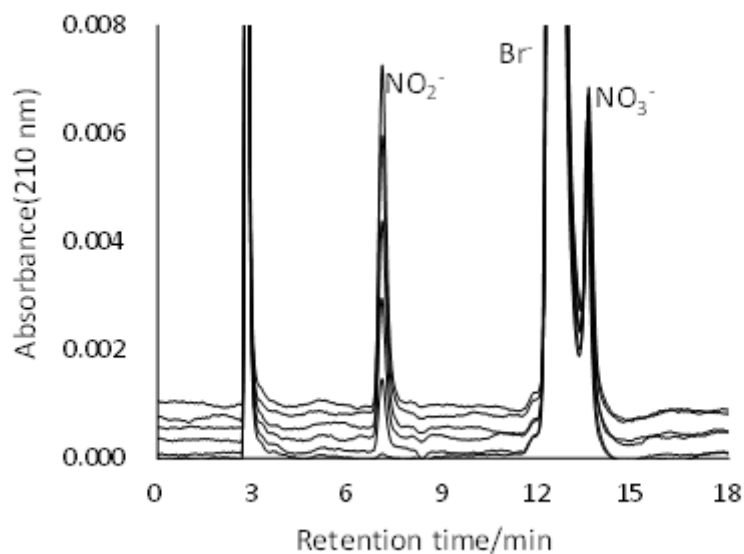


Figure 5

Chromatograms of seawater sample (MSW).

Pretreatment: only dilution to 0.20 g g^{-1} ; column: AS10; eluent: $80 \text{ mmol L}^{-1} \text{ NaCl}$; wavelength: 210 nm.

Chromatogram: (bottom) no addition of standard solutions; addition of 0.3 mg kg^{-1} nitrite for the largest