

# Evaluation of the Calibration Method for Accurate Analysis of Dissolved Silica by Continuous Flow Analysis

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For accurately determining nutrients in seawater by continuous flow analysis (CFA), the characteristic of the calibration curve was examined in detail. Under absorbance below 0.8, the calibration curve and the bracketing methods showed more accurate results than the bias fell below 0.5%. The analytical results of dissolved silica in seawater from the nutrient maximum layer of the Pacific Ocean obtained by the proposed methods showed good agreement with those obtained by an ion exclusion chromatography postcolumn absorption spectrophotometry (IEC-postcolumn) and an ion exclusion chromatography isotope dilution ICP mass spectrometry (IEC-ID-ICP-MS). From the results, the analysis of nutrients in seawater could be accurately carried out by CFA with an expanded uncertainty of below 1% using both the calibration curve and the bracketing methods with an appropriate absorbance range.

**Keywords** Seawater nutrients, dissolved silica, colorimetry, continuous flow analysis, calibration curve method, standard addition method, bracketing method

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## Introduction

Nutrients such as nitrate, nitrite, phosphate and dissolved silica in seawater are significantly important for animals and plants to maintain their biological activity.<sup>1-3</sup> In the field of oceanography and biochemistry, nutrients have been observed on a global scale for investigations of the food chain, material cycling in ecological systems and many environmental issues.<sup>1</sup> Therefore, we developed seawater certified reference materials (CRMs) for nutrient analysis (nitrate, nitrite, phosphate and dissolved silica) in 2014,<sup>4</sup> and the concentration of nutrients in the CRMs was attempted to be determined with a standard uncertainty of below 1% to contribute to an accurate analysis required in the field of oceanography and biochemistry. The 1% derives from the target precision proposed by the hydrographic observation protocols,<sup>1,2</sup> and the precision was determined based on the present situation concerning the differences between measurement laboratories (the standard deviation (SD) is 10% or more)<sup>5</sup> and the distribution of the nutrient fluctuation calculated by the  $\Delta C^*$  method, which is an estimation formula of anthropogenic carbon dioxide (for example, in the deep layer of the Pacific Ocean, the deviation of nitrate concentration, 10%, corresponds to 50% of the annual fluctuation of carbon dioxide concentration).<sup>6-8</sup> For the measurement of seawater nutrients, continuous flow analysis (CFA), based on colorimetry, is widely used, including on-board analysis; a calibration curve based on linear regression is commonly used. In our previous study,<sup>9</sup> the linearity of the

calibration curve for dissolved silica was examined with the molybdenum blue method by CFA; however, the calibration curve was fitted to non-linear regression rather than linear regression, which resulted in poor analytical result, since it was not perfectly linear. In order to obtain an accurate result, for example the standard uncertainty of below the 1%, batch mode by a spectrophotometer was also available because its calibration curve was fitted to linear regression. However, the batch operation is not practical because of being laborious and cumbersome, especially for on-board analysis. From this point of view, it is expected that the linearity of the calibration curve should be needed for CFA to achieve more accurate analysis, since CFA is useful for on-board analysis. Moreover, CFA has been recently applied to ISO of water quality<sup>10-12</sup> and determination using a calibration curve based on linear regression is commonly carried out. Furthermore, though there have been many examinations on CFA,<sup>13-17</sup> few papers have considered the uncertainty of the analytical result. Because the major component of the uncertainty for the analytical result is derived from the calibration curve, it is worth to clarify the characteristic of the calibration curve for CFA in detail.

In this study, three different calibration methods, such as calibration curve, standard addition and bracketing methods, were examined to analyze nutrients in seawater by CFA accurately, and their suitable concentration ranges were examined in detail. We focused on dissolved silica as one of the analytical targets of nutrients in seawater because the non-linearity of the calibration curve for dissolved silica had actually been reported.<sup>18,19</sup> In addition, from the viewpoint of the abundance rate in seawater, the requirement of accurate analysis for dissolved silica is higher than that for phosphate. In this study,

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Table 1 Major ionic compositions in seawater and ASW

	Average of world ocean <sup>22</sup> /g kg <sup>-1</sup>	ASW/ g kg <sup>-1</sup>
Cl <sup>-</sup>	19.35	18.75
Na <sup>+</sup>	10.78	12.20
Mg <sup>2+</sup>	1.28	0.68
SO <sub>4</sub> <sup>2-</sup>	2.69	2.67
HCO <sub>3</sub> <sup>-</sup>	0.14	0.12
Salinity	34 – 36	34.7

these calibration methods were applied to the analysis of dissolved silica in seawater, and the obtained analytical results, including their advantage or disadvantage, were discussed.

## Experimental

### Apparatus

A continuous flow analyzer AACS-V (BLTEC K.K., Osaka, Japan), which automatically carried out a color reaction followed by photometric detection, was employed for measurements of seawater nutrients.<sup>9</sup> The sample solutions were injected from an auto-sampler, in which a sample aliquot of 1.8 mL was stored in each vial covered by polytetrafluoroethylene (PTFE) septa. Measuring flasks, beakers and spoons used for the preparation of both reagent and measuring solutions were all made from polymethylpentene, polyethylene or PTFE. Storage bottles for both the reagent and the measuring solutions were made from polypropylene (PP).

### Reagents

All reagents were of analytical reagent grade available from FUJIFILM Wako Pure Chemical Corp. (Osaka, Japan) or Kanto Chemical Co. Inc. (Tokyo, Japan), unless otherwise specified. Water was purified using a Milli-Q Integral Q-POD Element system (Merck, Darmstadt, Germany). The silicon standard solution used was a Standard Reference Material (SRM) 3150 supplied by National Institute of Standards and Technology (NIST, Gaithersburg, USA). The standard solutions of nitrate were gravimetrically prepared by dissolving potassium nitrate (Kanto) in water. The purity of the salt was assayed by coulometric titration and gravimetric analysis.<sup>20</sup> Hereinafter, the concentration of dissolved silica and nitrate are identified based on the mass fractions (mg kg<sup>-1</sup>) of silicon (Si, atomic weight 28) and nitrate (NO<sub>3</sub><sup>-</sup>, molecular weight 62), respectively.

### Seawater sample

The seawater sample used in this study was NMIJ CRM 7603-a, commercially available from NMIJ/AIST (Tsukuba, Japan).<sup>4</sup> The CRM was based on the seawater collected from the nutrient maximum layer (3000 m depth) in the Pacific Ocean. Detailed descriptions of the sample are provided elsewhere.<sup>21</sup>

### Artificial seawater (ASW)

In this study, original artificial seawater (ASW) was prepared in accordance with the reference “Protocols for the Joint Global Ocean Flux Study (JGOFS) Core Measurements”<sup>21</sup> by dissolving 64.3 g of sodium chloride (NMIJ CRM 3008-a, NMIJ/AIST), 14.3 g of magnesium sulfate heptahydrate (Kanto) and 0.34 g of sodium hydrogen carbonate (Kanto) in 2000 mL of water at 25°C. The major ionic composition in both the average of the world ocean and the original ASW used in this study are listed

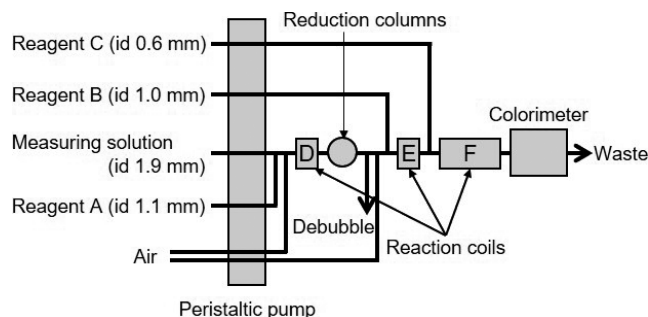


Fig. 1 Flow diagram for CFA. See the explanation in Table 2; “id” means the inner diameter of the tube to inject each solution.

in Table 1. The salinity was different, approximately from 34 to 36 g kg<sup>-1</sup>, depending on the ocean area, seasons and depth.<sup>22</sup> The concentration of each ion contained in the original ASW was similar to that in the average of the world ocean within ca. 10%, except for magnesium.

### Measurement by CFA

The measurement procedure of CFA was the same as that described in our previous paper.<sup>9</sup> The molybdenum blue method<sup>9,23,24</sup> was employed for the measurement of dissolved silica by the CFA. Three coloring reagents were prepared, as described in the literature:<sup>25</sup> (1) 0.06 mol L<sup>-1</sup> molybdate solution containing both 0.6 mol L<sup>-1</sup> sulfuric acid and 0.3% sodium dodecyl sulfate, (2) 0.40 mol L<sup>-1</sup> oxalic acid solution as a masking agent and (3) 0.14 mol L<sup>-1</sup> ascorbic acid solution. The Griess method<sup>26,27</sup> was employed for the measurement of nitrate. Nitrate was measured after its reduction to nitrite using a reduction column (Glastron, Inc., NJ, USA), which was made of coiled hollow cylindrical cadmium.<sup>28</sup> Three reduction columns were inserted in series in the CFA reaction line, as shown in Fig. 1. Complete conversion was allowed by three columns.<sup>29</sup> The following three solutions were prepared: (1) a 0.09 mol L<sup>-1</sup> imidazole solution as a catalyst containing both 0.02 mol L<sup>-1</sup> sulfuric acid and 0.1% Triton X-100, (2) a 0.06 mol L<sup>-1</sup> sulfanilamide solution as a modifier containing both 1.2 mol L<sup>-1</sup> hydrochloric acid and 0.1% Triton X-100, and (3) a 0.004 mol L<sup>-1</sup> N-1-naphthylethylenediamine dihydrochloride (1-NED) solution as a coupling agent containing 0.12 mol L<sup>-1</sup> hydrochloric acid. Note that, since nitrite was not contained in the nitrate solution used in this study, it was not necessary to subtract the nitrite by the Griess method. A flow diagram of CFA is shown in Fig. 1, and its supporting explanation is listed in Table 2; using these reagents freshly prepared, the analysis was carried out by CFA with the color reactions through a narrow tube.<sup>9</sup>

### Calibration methods for CFA

In this study, three different calibration methods, such as calibration curve, standard addition and bracketing methods, were applied to the determination of the nutrients in seawater. In the case of the calibration curve method, the calibration standard solutions of analyte added to ASW were used. In this study, except for the section “Calibration curve commonly-used by CFA”, the mass fraction of 0.04 g g<sup>-1</sup> seawater or ASW in the measuring solutions was adopted, which corresponded to 25 times dilution by water from the original seawater or ASW, in order to carry out the seawater analysis appropriately for dissolved silica in the Pacific Ocean. The concentration of the analyte in a seawater sample was calculated by the regression

Table 2 Detail of each item in the flow diagram

Analyte	Dissolved silica in water, ASW or seawater	Nitrate in water
Reagent A <sup>a</sup>	Molybdate solution <sup>c</sup>	Imidazole <sup>c</sup>
Reagent B <sup>a</sup>	Oxalic acid <sup>c</sup>	Sulfanilamide <sup>c</sup>
Reagent C <sup>a</sup>	Ascorbic acid <sup>c</sup>	1-NED <sup>c</sup>
Reaction coil D <sup>a</sup>	20 turns	5 turns
Reaction coil E <sup>a</sup>	5 turns	5 turns
Reaction coil F <sup>a</sup>	50 turns	15 turns
Reduction columns <sup>b</sup>	None	Inserted
Wavelength of colorimeter	550 nm	550 nm

a. The symbols (A to F) correspond to those in Fig. 1.

b. See Fig. 1.

c. Regarding each exact composition, see in text.

line obtained by plots of the absorbance from the calibration standard solutions. In the case of the standard addition method, the calibration standard solutions were prepared by adding the standard solution of the analyte to the seawater sample. The mass fraction of the seawater in the measuring solutions was 0.04 g g<sup>-1</sup> with 25-times diluted by water. The concentration of the analyte in the seawater sample was calculated by the *x*-intercept of the regression line from plots of the absorbance from the calibration standard solutions added. In the case of the bracketing method, two calibration standard solutions, which had higher and lower concentrations of analyte than that in seawater sample, were added to ASW. As similar to the calibration curve method, the mass fraction of the seawater or ASW in the measuring solutions was 0.04 g g<sup>-1</sup> when diluted by water (25-times dilution from original seawater or ASW). Using the regression line obtained by the two calibration standard solutions, the concentration of analyte in the seawater sample was calculated as follows:

$$x_{\text{smp}} = \frac{(x_{\text{H}} - x_{\text{L}})(y_{\text{smp}} - y_{\text{L}})}{y_{\text{H}} - y_{\text{L}}} + x_{\text{L}} \quad (1)$$

Here,  $x_{\text{smp}}$ ,  $x_{\text{H}}$  and  $x_{\text{L}}$  mean the concentration of a sample, and the higher and the lower calibration standard solutions;  $y_{\text{smp}}$ ,  $y_{\text{H}}$  and  $y_{\text{L}}$  mean the absorbance (or peak intensity) of a sample as well as the higher and the lower calibration standard solutions, respectively.

## Results and Discussion

### Calibration curve commonly-used by CFA

In the case of CFA, the calibration curve method with the matrix matched calibration standard solutions using ASW or surface seawater is commonly used.<sup>1</sup> An original seawater sample without dilution is commonly determined by the calibration curve with the concentration range of the nutrients, for example dissolved silica in 0–4 mg kg<sup>-1</sup>.<sup>1</sup> According to a previous study,<sup>9</sup> the calibration curve of dissolved silica by CFA was non-linear regression rather than linear regression. In the case of the calibration curve method, the linearity of the calibration curve is very important for accurate analysis. Therefore, we examined the linearity of the calibration curve, especially for dissolved silica in this study, as a function of the concentration range in 0–2 mg kg<sup>-1</sup>, which could be assumed as a common measurement. In addition, to check whether or not the trend of the calibration curve for the other nutrients is

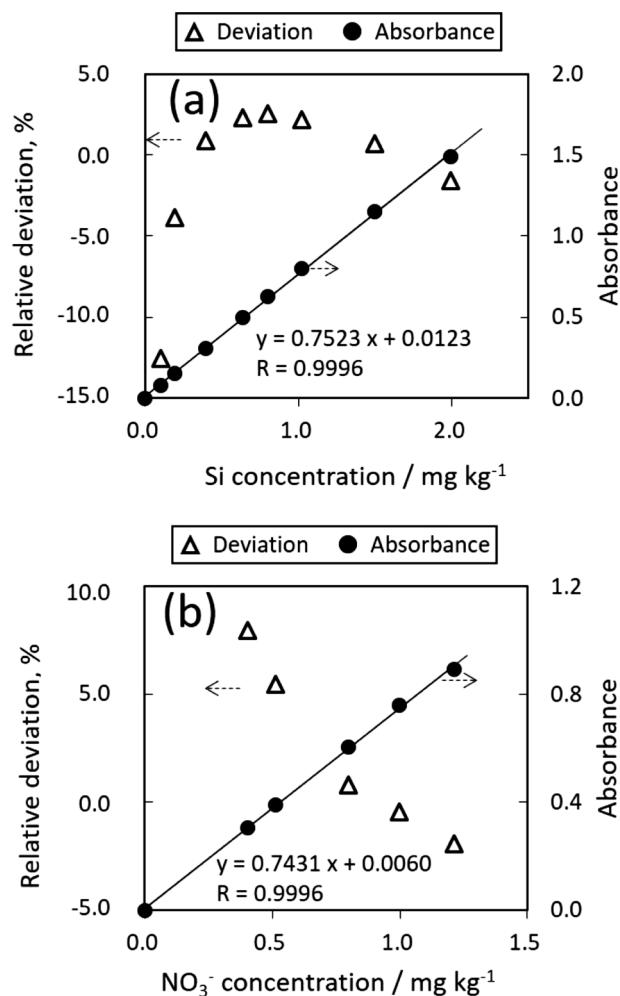


Fig. 2 Deviation between prepared and measured concentrations of the calibration standard solutions for (a) dissolved silica in water and (b) nitrate in water.

similar, nitrate was also examined as an example in addition to dissolved silica. The calibration curve of each nutrient was evaluated, and the deviations between the prepared concentrations and the measured concentrations, calculated by the regression line, are shown in Fig. 2. Although both of the calibration curves seemed to be linear, the deviations were not small ( $\pm 10\%$ ). This means that the calibration curves are not strictly linear, and the trend was observed for not only dissolved silica, but also for nitrate. Therefore, the trend seems to be typically observed for CFA. From these results, it was considered that a suitable concentration range of the calibration curve should be examined in advance for an accurate analysis of nutrients by CFA. The suitable concentration range might be related to the absorbance obtained by CFA. Note that, even though the measurement was carried out at these concentration ranges of the calibration curve shown in Fig. 2, the analytical result within an accuracy of  $\pm 10\%$  could be obtained. If the required accuracy is several %, the commonly-used calibration curve can be used in the concentration range mentioned above. However, if the accuracy required is below 1%, a suitable concentration range of the calibration curve should be used for CFA analysis.

### Examination of appropriate concentration and absorbance for calibration curve of CFA

According to the relative analytical error as a function of

transmission, the absorbance ranged from 0.1 to 1.0, which is acceptable for absorption spectrophotometry.<sup>30</sup> Therefore, the linearity of the calibration curve with absorbance below 0.8, which was below the acceptable absorbance of 1.0 as well as

corresponding to the concentration of dissolved silica below *ca.* 1 mg kg<sup>-1</sup> in either aqueous solution or ASW, was examined, as shown in Fig. 3. The deviations between the prepared concentrations and the measured concentrations calculated by the regression line were also evaluated. The mass fraction of ASW in Fig. 3(b) was 0.04 g g<sup>-1</sup>, which corresponded to 25-times dilution of seawater by water.

As can be seen from Fig. 3, each deviation between prepared and measured concentrations was approximately below 1.0%. This means that linear regression was possible for CFA in the absorbance range below 0.8. Therefore, the concentration and its absorbance of nutrients in seawater should be checked in advance, and the concentration should be prepared appropriately to obtain their absorbance at least below 0.8, in order to achieve accurate analysis by CFA. In addition, since a too low absorbance also gives a large analytical error, it is recommended that the minimum absorbance of the calibration curve be above 0.1, if possible.

#### Comparison of calibration methods

Based on the characteristic of the calibration curve for CFA, as described in the previous section, different calibration methods, such as standard addition and bracketing methods, were also studied in addition to the calibration curve one. For comparing three different calibration methods, each relative recovery rate (which means measured concentration/prepared concentration) with respect to 0.16 mg kg<sup>-1</sup> dissolved silica in 0.04 g g<sup>-1</sup> of ASW (25-times dilution) shown in Fig. 3, was evaluated by each calibration method with different concentration ranges; the analytical results are shown in Fig. 4.

As can be seen from Fig. 4, the standard addition method, which seemed to be more effective from a matrix matching point of view to achieve accurate analysis, gave a bias of above 1%, even though an added concentration range was appropriate, which corresponded to the absorbance below 0.8 observed by CFA. It was considered that the bias could be observed because the slope in the concentration range of 0 - 0.16 mg kg<sup>-1</sup> and that of 0.16 - 0.65 mg kg<sup>-1</sup> were different; the former and later slopes were 0.9221 and 0.9129, respectively. On the other hand, using the suitable concentration range, both the calibration curve and the bracketing methods could give an almost 100% recovery rate within  $\pm 0.5\%$ . From these results, it was considered that accurate analytical results could be obtained by both of the calibration methods, and the analytical performance was almost similar, even though the concentration of nutrients

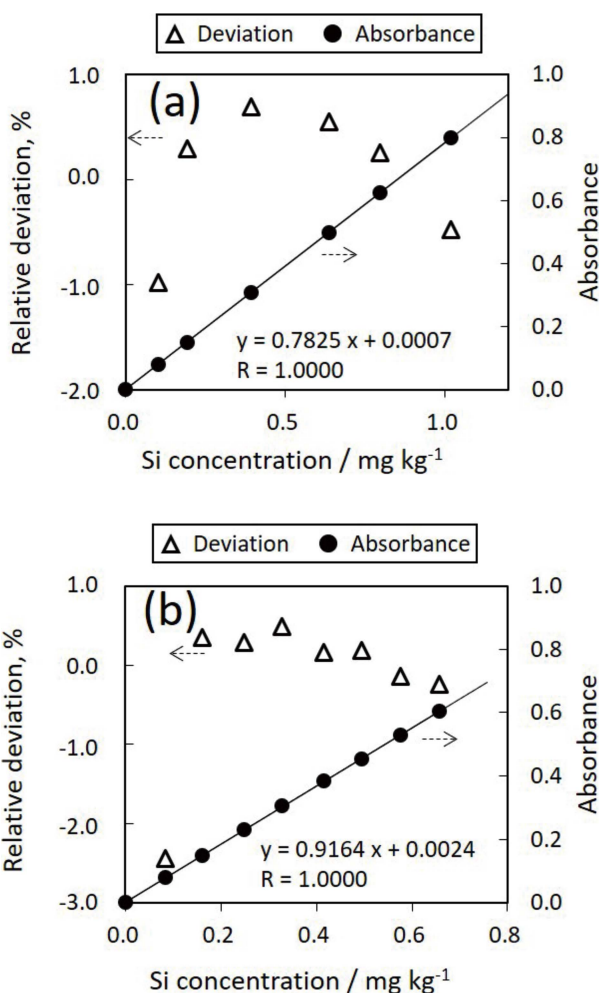


Fig. 3 Deviation between the prepared and measured concentration of the calibration standard solutions for dissolved silica in (a) water and (b) 0.04 g g<sup>-1</sup> of ASW (assuming seawater measurement).

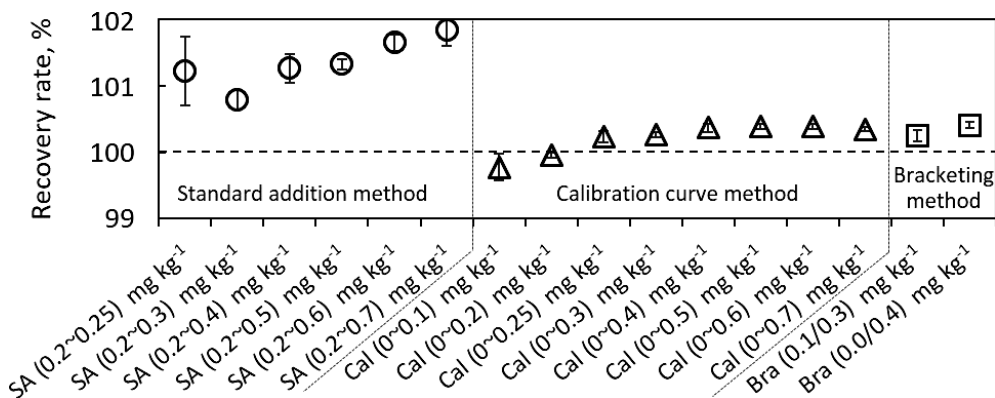


Fig. 4 Recovery rate of dissolved silica in 0.04 g g<sup>-1</sup> of ASW obtained by three different calibration methods. The concentration range of calibration standard solutions of dissolved silica is shown in parentheses and the bars attached with the plots show SD ( $n = 3$ ).

in seawater should be known in advance to prepare the calibration standard solutions with an appropriate concentration range. Therefore, the concentration range of the calibration curve and the bracketing methods should be carefully considered by a preliminary experiment to achieve accurate analysis with an uncertainty below 1%. Though both of the calibration methods showed a similar analytical performance, the bracketing method is expected to be labor saving, since the number of prepared standard solutions is less compared to that of the calibration curve method.

#### Examination on bracketing method

When the bracketing method is used, optimizing the concentrations of two calibration standard solutions should be considered. Using a measuring solution of  $0.04 \text{ g g}^{-1}$  of ASW containing  $0.16 \text{ mg kg}^{-1}$  of dissolved silica (same as section "Comparison of calibration methods") as a sample, the bracketing method was examined in detail using four standard solutions containing  $0.145$ ,  $0.153$ ,  $0.173$  and  $0.180 \text{ mg kg}^{-1}$  dissolved silica in  $0.04 \text{ g g}^{-1}$  of ASW (25 times dilution from original ASW) to carry out the bracketing  $\pm 5\%$ ,  $\pm 10\%$ ,  $-5$  to  $+10\%$  and  $-10$  to  $+5\%$  with respect to the sample concentration ( $0.16 \text{ mg kg}^{-1}$ ); the recovery rate obtained is shown in Fig. 5.

Using either the bracketing standard solutions of  $\pm 5\%$  or  $\pm 10\%$  with respect to the sample, the measured concentration agreed well with the prepared concentration with a bias within  $\pm 0.5\%$ . Since there is no statistical difference between the results of four combinations of the calibration standard solution sets, it could be evaluated that the bracketing method is available for the accurate analysis of nutrients in seawater by CFA when

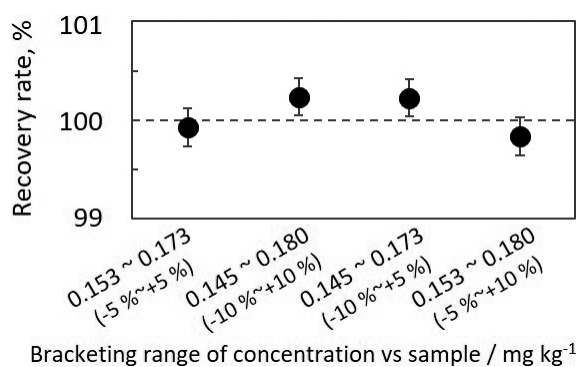


Fig. 5 Recovery rate vs. bracketing concentration of dissolved silica in  $0.04 \text{ g g}^{-1}$  of ASW. The bars attached with the plots shows SD ( $n = 10$ ).

a concentration range of  $\pm 10\%$  with an absorbance of below 0.8 is used. Therefore,  $\pm 5\%$  and  $\pm 10\%$  of the calibration standard solution sets were applied to the seawater nutrient analysis.

#### Analysis of seawater sample

The three different calibration methods for CFA mentioned in previous section were applied to the analysis of dissolved silica in seawater, NMIJ CRM 7603-a, which was collected from the nutrient maximum layer in the Pacific Ocean. The concentration of dissolved silica in the seawater was determined using the calibration curve, the standard addition and the bracketing methods. These results are shown in Table 3 and Fig. 6. Each analytical result of CFA means the average value of the measured concentrations of 10 bottles (each 4-times measurement). As listed in Table 3, the standard uncertainty of the analytical results of CFA was calculated by combining the following components: (1) the uncertainty due to the repeatability of the measurement, (2) the uncertainty due to the calibration curve, (3) the uncertainty due to the matrix difference between the sample and the standard solution sets and (4) the uncertainty of the standard solution. For the uncertainty of (1), the repeatability of SD,  $s_r$ , according to the ISO guide 35<sup>31</sup> or the

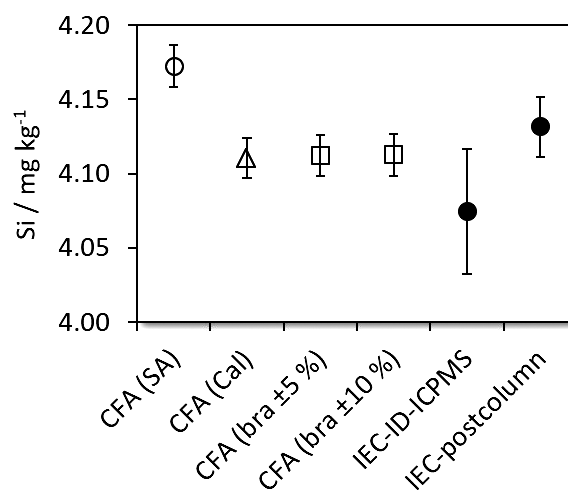


Fig. 6 Analytical results of dissolved silica in NMIJ CRM 7603-a. The different analytical methods of CFA, IEC-ID-ICPMS<sup>18</sup> and IEC-postcolumn<sup>17</sup> were applied for the analysis of dissolved silica, and three different calibration methods of the standard addition (SA), the calibration curve (Cal) and the bracketing methods (bra  $\pm 5\%$ ,  $\pm 10\%$ ) were used for CFA. The bars attached with the plots mean expanded uncertainty ( $k = 2$ ) of the analytical results.

Table 3 Analytical results and their uncertainty budgets for dissolved silica in NMIJ CRM 7603-a obtained by CFA with three calibration methods

Analytical results of dissolved silica in NMIJ CRM 7603-a (mass fraction/ $\text{mg kg}^{-1}$ )	Standard addition method	Calibration curve method	Bracket method ( $\pm 5\%$ )	Bracket method ( $\pm 10\%$ )
Average value $\pm$ combined standard uncertainty	$4.173 \pm 0.014$	$4.111 \pm 0.013$	$4.113 \pm 0.014$	$4.113 \pm 0.014$
Standard uncertainty (relative %)				
(1) Uncertainty due to the repeatability of five sample measurement	0.02	0.03	0.07	0.08
(2) Uncertainty due to the calibration curve	0.08	0.04	—	—
(3) Uncertainty due to matrix difference between sample and standard solutions	—	0.05	0.05	0.05
(4) Uncertainty of concentration of the Si standard solution	0.15	0.15	0.15	0.15
Combined standard uncertainty of sample concentration	0.17	0.16	0.17	0.17
Expanded uncertainty ( $k = 2$ )	0.34	0.33	0.34	0.35

SD of the average value was used. Using the calibration curve method, the uncertainty of (2) was calculated as following equations:<sup>32,33</sup>

$$u(x) = \frac{s}{a} \sqrt{1 + \frac{1}{n} + \frac{(y_0 - \bar{y})^2}{a^2 \sum (x_i - \bar{x})^2}} \quad (2)$$

When the standard addition method is used, Eq. (2) is deformed as follows:

$$u(x) = \frac{s}{a} \sqrt{1 + \frac{\bar{y}^2}{a^2 \sum (x_i - \bar{x})^2}} \quad (3)$$

where  $a$  is the slope of the standard addition calibration curve,  $s$  the residual SD from the calibration curve,  $n$  the total number of paired calibration points,  $x_i$  the concentration of dissolved silica (added to the sample solution) at each point  $i$  of the (standard addition) calibration curve;  $\bar{x}$  is the average of all  $x_i$  values,  $y_0$  the absorbance (or peak intensity) of the measuring solution  $\bar{y}$  and the average of the absorbance of all of the calibration points. Note that, in the case of the bracketing method, the uncertainty of (2) was not combined because it was contained in (1). Then, the uncertainty of (3) was combined to only the uncertainty of the calibration curve method and the bracketing one. The uncertainty of (3) was estimated according to a following experiment: after the silicon standard solution (NIST SRM 3150) was added to the seawater sample and ASW, the slope of the calibration curve in the same absorbance range were compared. The deviation between the slopes of the calibration curve based on the seawater sample and that on ASW (0.05%) was adopted as the uncertainty (3), when both calibration standard solution sets had a mass fraction of 0.04 g g<sup>-1</sup> seawater or ASW. Finally, the standard uncertainty of silicon standard solution (NIST SRM 3150, 0.15%) was used for the uncertainty of (4).

Although all of the results of CFA were obtained using the calibration standard solution sets at the suitable absorbance range, as shown in Fig. 6, the standard addition method gave the result with bias of over 1%. Meanwhile, the results of the calibration curve and the bracketing methods agreed with each other. Under a suitable concentration range as well as absorbance, it was indicated that these two methods could give an equivalent result.

The analytical results by CFA were also compared with that by an ion exclusion chromatography (IEC) isotope dilution ICP mass spectrometry (IEC-ID-ICPMS)<sup>21</sup> and IEC postcolumn absorption spectrophotometry (IEC-postcolumn).<sup>17</sup> They have a different principle of separation and/or detection from that of CFA. As shown in Fig. 6, the analytical results of CFA using the calibration curve and the bracketing methods agreed well with those of IEC-ID-ICPMS and IEC-postcolumn within each expanded uncertainty ( $k = 2$ ). This also indicates that ASW can be used for a matrix matching solution of seawater, for example the Pacific Ocean. By comparing the uncertainties of each analytical methods (refer to Table 3) and previous papers,<sup>17,21</sup> it was confirmed that CFA showed a better repeatability than the others. Therefore, CFA allowed measurement with less uncertainty compared to the other analytical methods. In addition, it was shown that CFA could give an equivalent result to that of IEC-ID-ICPMS, which was one of the primary standards of measurement that involves a method having the highest metrological qualities.<sup>34</sup> From these results, it was considered that the calibration curve and bracketing methods by CFA were effective for an accurate analysis of dissolved silica, and the ASW could be used for a matrix matching solution for calibration standard solutions.

## Conclusions

The characteristic of the calibration curve of CFA was examined in detail for accurate analysis of dissolve silica in seawater. In case of measurement using the calibration curve whose absorbance range was up to 2 (like a measurement of non-diluted seawater with high concentration of the nutrients in the Pacific Ocean *etc.*), the calibration curve did not fit strictly to linear regression, though a determination with an uncertainty of several % was possible. However, if more accurate analysis is required, the calibration curve with an absorbance range of 0.1 - 0.8 is at least necessary for CFA. Using the calibration curve with the absorbance range, the deviation between the prepared and measured concentrations obtained by the regression line was below 1%, since the calibration curve could provide good linearly.

Under the suitable absorbance range of 0.1 - 0.8, three different calibration methods were applied to the analysis of dissolved silica in real seawater, NMIJ CRM 7603-a. However, when using the standard addition method, a bias of approximately 1% was observed, even though a suitable absorbance range was used. Meanwhile, both the calibration curve and the bracketing methods gave equivalent results for the analysis of dissolved silica. In addition, the results of these two calibration methods by CFA agreed well with those of IEC-ID-ICPMS and IEC-postcolumn absorption spectrophotometry. The bracketing method has advantages that the operation of the preparation is simpler and the measurement time is shorter than that of the calibration curve method, though the analytical results for both methods were not different. From these results, it was evaluated that ASW could be used as the matrix matching solution with respect to seawater samples from the Pacific Ocean, and the analysis of the nutrient in seawater samples could be accurately carried out by CFA with the expanded uncertainty below 1% using both the calibration curve and the bracketing methods with an appropriate concentration range, even though the concentration range should be examined in advance.

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