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$^{44}\text{Ca}/^{43}\text{Ca}$   
Isotope dilution  
ID-ICP-MS

Ca

## Seawater Calcium

ISSN 0003-2654



PAPER

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Precise determination of seawater calcium using isotope dilution inductively coupled plasma mass spectrometry

# Precise determination of seawater calcium using isotope dilution inductively coupled plasma mass spectrometry

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We describe a method for rapid, precise and accurate determination of calcium ion ( $\text{Ca}^{2+}$ ) concentration in seawater using isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS). A 10  $\mu\text{L}$  aliquot of seawater was spiked with an appropriate  $^{43}\text{Ca}$  enriched solution for  $^{44}\text{Ca}/^{43}\text{Ca}$  ID-ICP-MS analyses, using an Element XR (Thermo Fisher Scientific), operated at low resolution in E-scan acquisition mode. A standard-sample bracketing technique was applied to correct for potential mass discrimination and ratio drift at every 5 samples. A precision of better than 0.05% for within-run and 0.10% for duplicate measurements of the IAPSO seawater standard was achieved using 10  $\mu\text{L}$  solutions with a measuring time less than 3 minutes. Depth profiles of seawater samples collected from the Arctic Ocean basin were processed and compared with results obtained by the classic ethylene glycol tetra-acetic acid (EGTA) titration. Our new ID-ICP-MS data agreed closely with the conventional EGTA data, with the latter consistently displaying 1.5% excess  $\text{Ca}^{2+}$  values, possibly due to a contribution of interference from  $\text{Mg}^{2+}$  and  $\text{Sr}^{2+}$  in the EGTA titration. The newly obtained Sr/Ca profiles reveal sensitive water mass mixing in the upper oceanic column to reflect ice melting in the Arctic region. This novel technique provides a tool for seawater  $\text{Ca}^{2+}$  determination with small sample size, high throughput, excellent internal precision and external reproducibility.

Received 16th October 2013  
Accepted 21st December 2013

DOI: 10.1039/c3an01948a

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## 1. Introduction

Calcium ion ( $\text{Ca}^{2+}$ ) is one of the most important major cations in seawater and is closely involved in the oceanic carbonate system, which is sensitive to variations in global carbon recycling among atmosphere-oceans-continent for forcing climate and anthropogenic changes.<sup>1-4</sup> The nature of the mechanism that controls  $\text{Ca}^{2+}$  distribution in seawater is, therefore, a subject of major interest in chemical oceanography and carbon cycling research. For example, the imbalance of  $\text{Ca}^{2+}$ , that river input is only one half of sea floor removal, has been a long standing puzzle in geochemistry of the ocean.<sup>5</sup> Most recently, the increase in oceanic uptake of the anthropogenic  $\text{CO}_2$  from the atmosphere certainly acidifies seawater and potentially harms the health of marine calcifying organisms.<sup>6,7</sup> Thus, a new

need for effective analytical tools for seawater  $\text{Ca}^{2+}$  analysis surged as a result of the enormous societal concern on and the intense scientific interest in ocean acidification.

High precision  $\text{Ca}^{2+}$  determination techniques are essential for performing sensitive investigations of carbon cycle related problems in oceanography as the background concentration of  $\text{Ca}^{2+}$  is high in ocean water ( $\sim 10$  mM) and the degree of  $\text{Ca}^{2+}$  variation throughout the ocean water column is typically rather small, only on a scale of several percent or less.<sup>8-10</sup> Over the past few decades, determination of  $\text{Ca}^{2+}$  in seawater by various titration techniques using ethylene glycol tetra-acetic acid (EGTA) has become well established, achieving a precision of  $\sim 0.1\%$ <sup>11-14</sup> in relative standard deviation (RSD). However, these titration techniques have faced two major challenges in terms of analytical efficiency and improvements in potential interference.<sup>15,16</sup> Potential interference by  $\text{Mg}^{2+}$  and  $\text{Sr}^{2+}$  in  $\text{Ca}^{2+}$  titration is an important hurdle in improving the accuracy in EGTA, especially in cases where unsuitable electrodes are chosen or high  $\text{Mg}^{2+}$  and/or  $\text{Sr}^{2+}$  samples.<sup>15-17</sup> The large amount of sample and the long analytical time by titration procedures, typically requiring at least 20 mL and 20 minutes for each individual measurement, are also serious obstacles for many other applications that require higher analytical efficiency and more savings of sample consumption. Consequently, it is critical to develop new  $\text{Ca}^{2+}$  analytical techniques to meet the

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demands for many potential applications in oceanography and geochemistry.

Isotope dilution mass spectrometry (ID-MS) is a well-established analytical technique based on determining the isotope ratios in an analyte where its isotopic composition results from an appropriate mixture of the original sample and a known selected isotope-enriched spike standard.<sup>18,19</sup> ID-MS generally provides the highest accuracy and precision of any available chemical calibration method, as it is based on a high precision isotopic determination rather than an elemental intensity calibration.<sup>18</sup> In fact, ID has been applied in thermal ionization mass spectrometry (ID-TIMS) and provided high quality analysis with 0.01% RSD.<sup>18</sup> In seawater, Ca<sup>2+</sup> measurements by ID-TIMS with 0.05% precision have been reported, with the sample size reduced to 1 g.<sup>8</sup> But its lengthy and complicated preparation procedures, as well as its high cost and extensive labor involvement, prevent it from becoming a routine analytical method. Recent attention to ocean acidification research and Arctic Ocean carbon cycle research further highlight the demands for high throughput and small volume analysis of Ca<sup>2+</sup> in seawater and biological samples.<sup>20,21</sup> The above mentioned shortcomings can be overcome by the strengths of sector field inductively coupled plasma mass spectrometry (SF-ICP-MS), which has the advantages of rapid isotopic ratio scan, extremely low detection limit, high sensitivity and selectivity, and no matrix artifacts. To date, ID-ICP-MS has only found limited applications for determining trace metals in seawater, *i.e.* Fe,<sup>22</sup> Cr,<sup>23</sup> and Cd,<sup>24</sup> achieving a precision of approximately 0.05–0.2% RSD.<sup>22–24</sup> This study aims, for the first time, to establish and evaluate a high precision ID-ICP-MS procedure for a major element analysis, that is Ca<sup>2+</sup> determination, in seawater, with small sample size, high throughput, and excellent external reproducibility.

## 2. Experimental

### Reagents and materials

In order to reduce the chemical blank and to eliminate potential interference in the Ca isotope determination, all experiments were conducted inside a class-1000 clean room at the Earth Dynamic System Research Center (EDSRC) at the National Cheng Kung University (NCKU), Tainan, Taiwan. The sample pretreatments were performed under a class-10 clean airflow working bench. In addition, HNO<sub>3</sub> and HCl were purified from GR grade reagents by double sub-boiling distillation and 18.2 ΩM grade Milli-Q water was prepared using an Elix 5 system (Millipore, Billerica, MA, USA).

An enriched <sup>43</sup>Ca isotopic standard purchased from Oak Ridge National Laboratory (ORNL, Oak Ridge, TN, USA) was used for the <sup>44</sup>Ca/<sup>43</sup>Ca ID-ICP-MS in this study. A working spiked solution of 10 μg g<sup>-1</sup> Ca<sup>2+</sup> was prepared by dilution with 5% HNO<sub>3</sub>. The concentration of the spiked solution was firstly estimated using a calibrated <sup>43</sup>Ca enriched standard provided by the Institute of Earth Sciences, Academia Sinica in Taipei, Taiwan. The precise concentration of the spike solution was further calibrated by a reverse ID-ICP-MS analysis.

A seawater standard, IAPSO (OSIL, Hampshire, United Kingdom, Batch #P137), was used as a reference material for analytical protocol validation and direct comparison with available literature data. Other natural seawaters analyzed in this study consisted of two sets of specimens collected in September 2012 (77.50° N, 172.00° W and 76.50° N, 172.00° W) during a cruise to the Arctic Ocean. Twenty-five seawater samples, comprising two vertical profiles from the surface to a depth of 2200 m, were selected and Ca ID-ICP-MS determination was performed. These samples were acidified to pH 2 with twice sub-boiling concentrated HCl acid (~9 N) and stored at 4 °C before chemical analysis.

### Sample preparation

An optimum sample/spike ratio is critical for achieving high precision isotopic ratio analyses by ID-ICP-MS. The error propagation factor can be evaluated, mainly based on the differences in isotopic abundances between the natural samples to be spiked and the spike reagent. The optimum exact theoretical error propagation factor (EPF<sub>opt</sub>) can be estimated from the following formula:<sup>25</sup>

$$\begin{aligned} \text{EPF}_{\text{opt}} &= \sqrt{\frac{\text{Main spike abundance}}{\text{Minor spike abundance}} \cdot \frac{\text{Minor sample abundance}}{\text{Main sample abundance}}} \\ &= \sqrt{\frac{\%43_{\text{S}}}{\%44_{\text{S}}} \cdot \frac{\%43_{\text{N}}}{\%44_{\text{N}}}} \end{aligned} \quad (1)$$

where the subscripts “N” and “S” denote the items for natural seawater (unknown sample) and the spiked standard, respectively, and “%” is the corresponding isotopic abundance. The estimated optimum <sup>44</sup>Ca/<sup>43</sup>Ca ratio for isotopic determination is 0.95. Thus, a 10 μL sample of seawater was spiked with 10 mg of 10 μg g<sup>-1</sup> <sup>43</sup>Ca spike solution, and then diluted to a final weight of 2.5 g using purified 5% HNO<sub>3</sub>. All the samples and reagents were weighed precisely using a six-digit analytical balance. All samples were confirmed to be in a well-mixed state after ultra-sonication for at least 30 min and stored over night before ID-ICP-MS determination.

### Instrumentation and methodology

All measurements of <sup>44</sup>Ca/<sup>43</sup>Ca isotopic ratios were carried out using a SF-ICP-MS (Element XR, Thermo Fisher Scientific, Germany) at NCKU. In the present study, the instrument was operated under low resolution and in E-scan acquisition mode (electrostatic). Daily optimization of the instrument was conducted after a mass calibration check, as recommended by the manufacturer, using a 1 ng g<sup>-1</sup> multi-element standard solution to tune for the best sensitivity and stability. Then, IAPSO seawater spiked with a <sup>43</sup>Ca enriched standard was used as a second monitoring standard to further ensure the <sup>44</sup>Ca/<sup>43</sup>Ca stability.

Rapid scans of peak flat top of signals for <sup>43</sup>Ca and <sup>44</sup>Ca were performed and 3000 runs of each isotope were integrated. All data acquisition was completed within 3 minutes. A data reduction procedure was carried out off-line after blank

Table 1 Typical operation conditions and parameters

RF power	1200 W
Guard electrode	On
Plasma Ar gas flow rate	15.0 L min <sup>-1</sup>
Auxiliary Ar gas flow rate	0.75 L min <sup>-1</sup>
Ar carrier gas flow rate	1.0–1.03 L min <sup>-1</sup>
Cones	Sampler: nickel, 1.1 mm/skimmer: nickel, 0.8 mm
Sample uptake rate	100 μL min <sup>-1</sup>
Extraction voltage	2 kV
Accelerating voltage	10 kV
Mass resolution	Low mass resolution mode (~300)

subtraction using spreadsheet programs. A data filtering process was applied to the 3000 runs data collected, from which outliers were removed to approach 1 RSD of the raw data.

Mass discrimination artifacts (mass bias effect) and low-frequency ratio drift in ICP-MS may bias the measured isotope ratios from the true values significantly. We used IAPSO seawater as the bracketing standard to correct for any mass discrimination and ratio drift at every 5 samples. These corrections were done based on a linear law. The typical instrument settings and operation parameters are summarized in Table 1.

### Calculation of isotope dilution

A general equation for quantitative interpretation of ID results was derived from the following expression:<sup>26</sup>

$$C_N = \text{At. wt}_N \cdot \frac{\%44_S - R\%43_S}{R\%43_N - \%44_N} \cdot \frac{\text{wt}_S}{\text{wt}_N} \cdot \frac{C_S}{\text{At. wt}_S} \quad (2)$$

where “ $C_N$ ” and “ $C_S$ ” are the concentration of Ca in the unknown natural seawater samples and the processed spiked standard, respectively. “ $R$ ” is the mass discrimination corrected  $^{44}\text{Ca}/^{43}\text{Ca}$  ratio, “%” is the isotopic abundance, “At.” is the atomic weight, and “wt” is the weight of the added seawater (N) or the added spiked (S) solution. The Ca isotopic abundance in the spike standard was adopted from the isotopic assay calibrated by Oak Ridge National Laboratory (Batch: 169191), and in the natural samples based on the International Union of Pure and Applied Chemistry (IUPAC) Technical Report.<sup>27</sup>

## 3. Results and discussion

### Spectral interference and blanks

An insignificant isobaric bias for the measured isotopes is a fundamental requirement for achieving accurate isotopic determination by ID-ICP-MS. The potential spectral interference for  $^{44}\text{Ca}/^{43}\text{Ca}$  determination is molecular interference by  $^{14}\text{N}_3^1\text{H}^+$  on  $^{43}\text{Ca}$ ,  $^{12}\text{C}^{16}\text{O}_2$  and  $^{14}\text{N}_2^{16}\text{O}^+$  on  $^{44}\text{Ca}$ , and doubly-charged  $^{86}\text{Sr}^{2+}$  and  $^{88}\text{Sr}^{2+}$  on  $^{43}\text{Ca}$  and  $^{44}\text{Ca}$ , respectively. To reduce these types of potential molecular interference, we kept the signals for  $^{43}\text{Ca}$  and  $^{44}\text{Ca}$  as high as possible. As a result, background contributions were <0.02% for  $^{43}\text{Ca}$  and <0.43% for  $^{44}\text{Ca}$  compared with the typical measured signals of the spiked IAPSO standards, and could be corrected directly using the

adjacent blank measurement in the analytical sequences. The doubly charged ions could also bias the  $^{44}\text{Ca}/^{43}\text{Ca}$  ratio measurements. In the present study, we use a high purity Sr standard solution with a concentration similar to the content in seawater for an interference assessment of Sr doubly charged ions on Ca isotope determination. In our instrument, doubly charged ion formation was evaluated at ~3%, and contributed less than 0.05% compared to the integrated signals of the Ca isotopes. Therefore, the double-charged ions are negligible with respect to Ca isotope determination. Thus, only left molecular interference at  $^{44}\text{Ca}/^{43}\text{Ca}$  needed to be measured and required for correction.

### Data acquisition parameters

For further evaluation of the optimum conditions that could be applied for precise  $^{44}\text{Ca}/^{43}\text{Ca}$  determination, various data acquisition parameters were examined carefully using the spiked IAPSO standard. In this study, the settling time and numbers of scans were set at 0.001 s and 3000 runs, respectively. Mass windows and numbers of samples per peak were selected to achieve the most precise isotopic determination. Reducing the mass windows from 20 to 5% coupled with adjusting the numbers per peak between 50 and 200 were evaluated. Taking both analytical quality and efficiency into consideration, 5% mass windows and 120 samples per peak were selected. The optimum parameters for data acquisition are summarized in Table 2. Under the selected conditions, the spectrum presented a flat-topped signal and only the center of the peak region was integrated. Data reduction was performed to improve the analytical precision of the ICP-MS isotopic ratios by removing statistical outliers. Data differences by more than 1 standard deviation from the mean value were identified as outliers. Overall, more than 67% (2000 runs) of data passed the filtering process and the RSD could be improved to better than 0.03% in most cases.

### Mass discrimination

In our analytical protocol, the IAPSO seawater standard was utilized as a bracketing standard to monitor the instrumental sensitivity, stability and ratio drift in the entire analytical sequence. Since a well-matched matrix standard was used and precise weighing was controlled for sample dilution, matrix and intensity bias artifacts were rather insignificant for the analyzed

Table 2 Optimum data acquisition parameters for  $^{44}\text{Ca}/^{43}\text{Ca}$  isotopic determination

Acquisition mode	E-scan (electrostatic)
Detector mode	Analog mode only
Mass window	5
Sample per peak	120
Settling time	0.001 s
Run/pass	3000/1 (off line data filtering: outlier of 1 SD)
Analysis time	<3 min
Sample size	<500 μL (take-up time and stabilization time included)



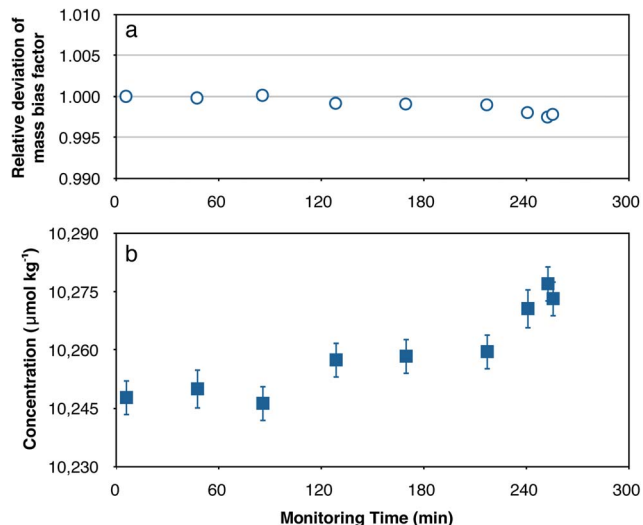


Fig. 1 Mass bias (a) and processed [Ca] drift (b) over approximately 4-hour measurement experiment.

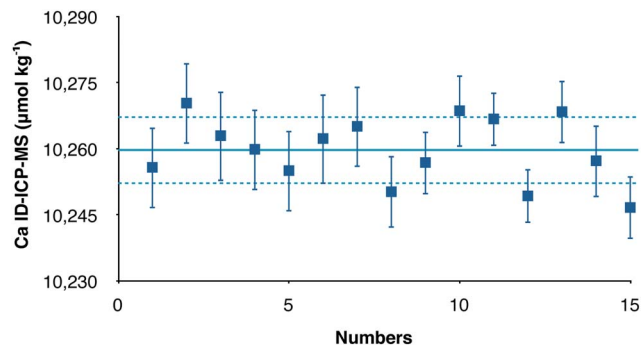


Fig. 2 Replicate measurements of standard seawater IAPSO using the proposed analytical procedures over a period of 4 months (Nov, 2012 to Feb, 2013 with 5 analytical days). Solid lines are the mean values of determined [Ca] and dashed lines for errors represented as 1 standard deviation.

samples and the standards. Because mass discrimination may seriously limit analytical precision and accuracy, the mass bias drift factor (i) was obtained by a linear law:<sup>28</sup>

$$R_{\text{calculated}} = iR_{\text{measured}} \quad (3)$$

In this study, the magnitude of mass discrimination varied daily, averaging 8.8% for  $^{44}\text{Ca}/^{43}\text{Ca}$  determination by comparison of the estimated isotopic ratio according to the precise weighing calculation and the measured ratio in the IAPSO standard. Fig. 1 presents a series of IAPSO measurements over a 4 h monitoring period in a single day to evaluate the relative mass bias drift and the degree of corrections for seawater  $\text{Ca}^{2+}$  concentration. As shown, no significant drift in the mass bias factor or  $\text{Ca}^{2+}$  concentration was observed within the first 2 h. Then, slight drifting occurred, which remained stable over the second 2 h, and the stability deteriorated gradually after 4 h. This indicates the mass discrimination correction functioning well during the first 4 h of daily measurement. The deteriorative stability after long operation hours is usually dominated by changes in plasma stability or cone condition. Furthermore, any small scale of instability in mass discrimination may potentially cause drifts in mass bias corrected  $^{44}\text{Ca}/^{43}\text{Ca}$  ratio and further result in certain biases on the absolute  $\text{Ca}^{2+}$  value of samples. An approximately 0.05% increase per hour was detected in the daily measurement (4 hours). Therefore, a standard-sample-standard bracketing method was applied at every 5 samples for mass discrimination and ratio drift correction in this study.

### Total uncertainty budget

A detailed budget of potential uncertainties was compiled in Table 3 to include all parameters considered in eqn (2) for evaluating potential errors in the Ca ID-ICP-MS measurement<sup>24,29</sup> (Table 3). The main error in the list is predominated by the abundance calibration of  $^{43}\text{Ca}$  and  $^{44}\text{Ca}$  nuclides in the spike (up to 1.48%). But the accuracy of Ca isotopic abundances in spiked solutions can be further improved by the reverse ID-ICP-MS procedure, and the correction factor was taken into account by a combined value of mass bias factor and corrected by the mass discrimination effect in the isotopic determination. Thus, possible random errors were contributed by experimental operations including weighing practice, calibration of the spike reagent, reverse calibration by ID-ICP-MS, and mixture of isotopic ratios. The sum of uncertainties, evaluated using IAPSO

Table 3 Total uncertainty budget for Ca ID-ICP-MS determination in seawater matrix

Parameter	Symbol <sup>c</sup>	Unit	Value	SD	RSD
Abundance of $^{43}\text{Ca}$ spike <sup>b</sup>	%43 <sub>S</sub>	%	83.93	0.1	0.12% <sup>d</sup>
Abundance of $^{44}\text{Ca}$ spike <sup>b</sup>	%44 <sub>S</sub>	%	5.06	0.03	0.59% <sup>d</sup>
Abundance of $^{43}\text{Ca}$ sample <sup>c</sup>	%43 <sub>N</sub>	%	0.135	0.002	1.48% <sup>d</sup>
Abundance of $^{44}\text{Ca}$ sample <sup>c</sup>	%44 <sub>N</sub>	%	2.086	0.004	0.19% <sup>d</sup>
$^{44}\text{Ca}/^{43}\text{Ca}$ (mass bias corrected)	<i>R</i>				0.05%
Concentration of spike (reversed ID)	<i>C<sub>S</sub></i>	ppm	10	0.0088	0.05%
Weight of spike	wt <sub>S</sub>	g	0.01	0.000016	0.16%
Weight of sample	wt <sub>N</sub>	g	0.01	0.000011	0.11%
Weight of reagent		g	3.5	0.000012	0.0003%
Combined uncertainty					0.21%

<sup>a</sup> Symbols used in eqn (2). <sup>b</sup> Data obtained from the assay report of  $^{43}\text{Ca}$  standard (Batch: 169191) by Oak Ridge National Laboratory. <sup>c</sup> Data obtained from Coplen *et al.* (2002).<sup>27</sup> <sup>d</sup> The parameter uncertainties are corrected by the calibration of the reversed ID procedure, and accounted into the combined uncertainty of mass bias factor.

seawater, can be improved to a value of 0.2% (RSD). The most important error was contributed by the weighing practice. The integrated uncertainties generated from the concentration of the spike reagent, reverse calibration by ID-ICP-MS, and the isotopic ratio determination of the mixture typically had a precision of approximately 0.05%, rather excellent compared with other available methods.

### Accuracy, precision and reproducibility

Daily or freshly prepared aliquots of IAPSO, rather than the monitoring standard, were used to assess the uncertainties associated with our long-term operation. This stock solution was also used for QA/QC assurances in daily measurement and for long-term reproducibility evaluation. Our Ca ID-ICP-MS results of  $10\,255 \pm 10 \mu\text{mol kg}^{-1}$  (SD,  $n = 15$ , RSD = 0.1%)

obtained over a 4-month period (Fig. 2), are in excellent agreement with the high precision EGTA data ( $10\,260 \pm 6 \mu\text{mol kg}^{-1}$ ).

### Applications and analytical validation

Applying the above-mentioned procedures, we analyzed two sets of seawater samples collected from the Arctic Ocean basin (a polar-ward station site #1:  $77.50^\circ \text{N}$ ,  $172.00^\circ \text{W}$  and a continental-ward station site #2:  $76.50^\circ \text{N}$ ,  $172.00^\circ \text{W}$ ). The ID-ICP-MS  $\text{Ca}^{2+}$  increased from  $7845 \mu\text{mol kg}^{-1}$  in shallow water to a value of  $10\,321 \mu\text{mol kg}^{-1}$  near the bottom of the ocean at site #1, as well as values of  $7270 \mu\text{mol kg}^{-1}$  to  $10\,026 \mu\text{mol kg}^{-1}$  at site #2 (Fig. 3b). While the full geochemical interpretation will be given elsewhere (W.-J. Cai, pers. communication), briefly, those profiles reflect a surface dilution by sea-ice melting and river fluxes. These differentiated dilution effects resulted in a

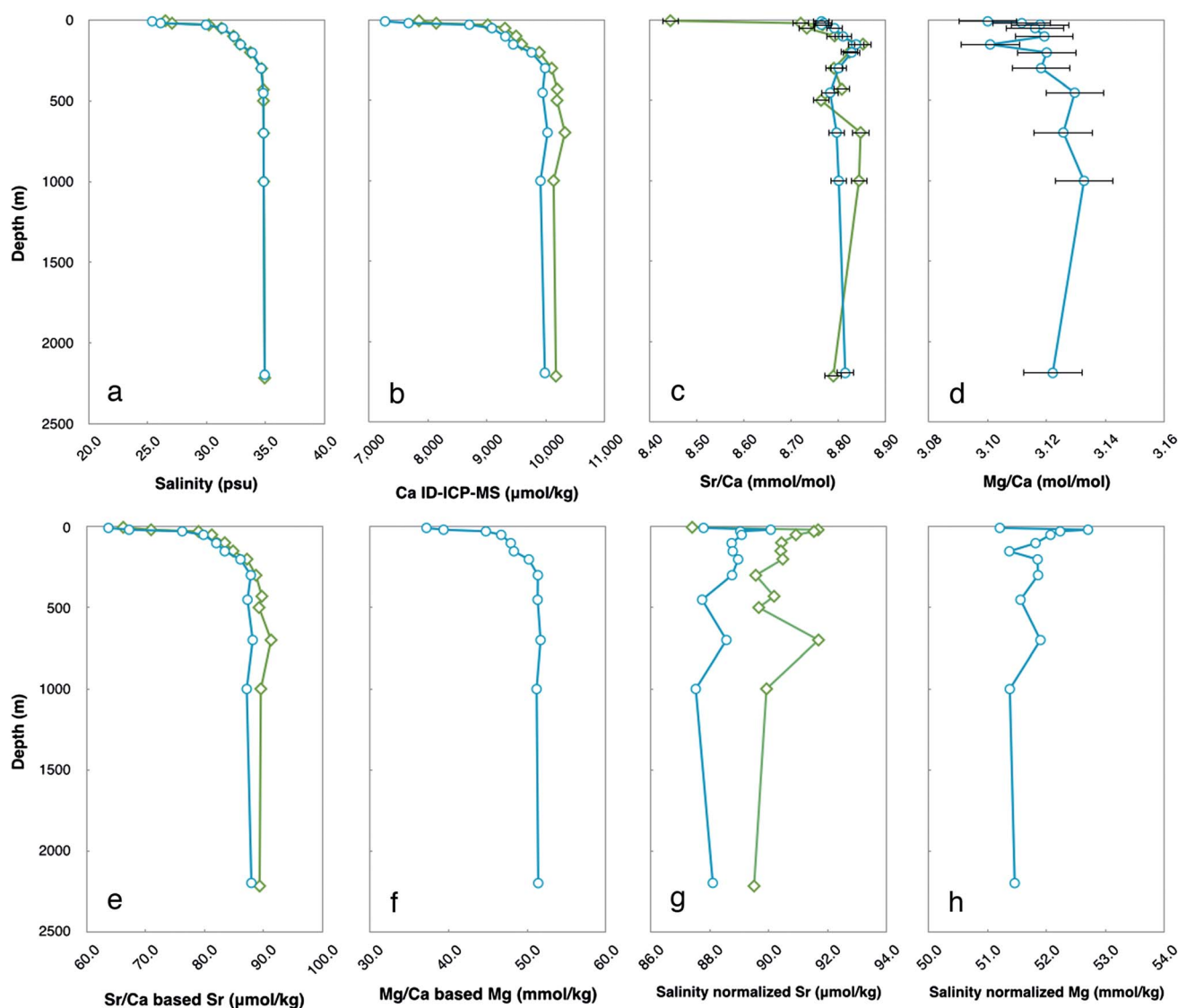


Fig. 3 Profile distribution of collected seawater sets: (a) salinity, (b) ID-ICP-MS  $\text{Ca}^{2+}$ , (c) Sr/Ca ratio, (d) Mg/Ca ratio, (e) Sr/Ca ratio based Sr concentration, (f) Mg/Ca ratio based Mg concentration, (g) salinity normalized Sr content and (h) salinity normalized Mg content (diamond for  $77.50^\circ \text{N}$ ,  $172.00^\circ \text{W}$  and point for  $76.50^\circ \text{N}$ ,  $172.00^\circ \text{W}$ ). The normalization was based on diagram (a), and calculated to the salinity of 35 psu (or = metal/S  $\times$  35).

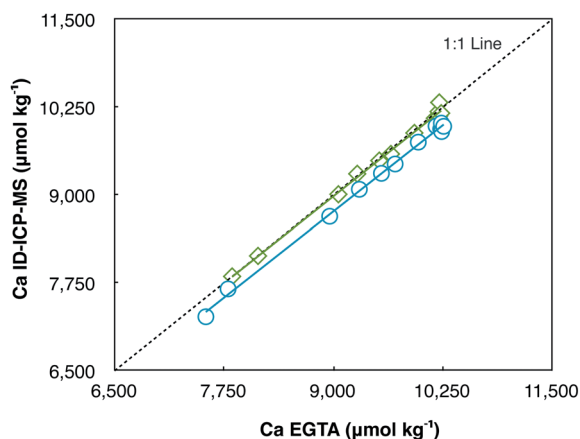


Fig. 4 Inter-calibration of the Arctic Ocean seawater set Ca concentration processed by Ca ID-ICP-MS and EGTA techniques (diamond for 77.50° N, 172.00° W and point for 76.50° N, 172.00° W).

difference in  $\text{Ca}^{2+}$  values between the profiles, particularly at the surface water. In fact, the Arctic water is formed by a less-salty Pacific water at to most 150 m and more saline Atlantic water at below.<sup>30</sup> To further validate our new analytical protocol, the same sets of seawater samples were analyzed using EGTA titration, mainly based on a protocol proposed by Lebel and Poisson (1976).<sup>17</sup> The  $\text{Ca}^{2+}$  concentrations obtained by ID-ICP-MS and EGTA titration agreed closely with each other. The classic Pearson's correlation (PC) was computed using SPSS 17.0 to examine the concentration obtained by the two techniques, and suggested a significant correlation between the two datasets ( $r = 0.992$ ,  $p = 0.00$ ,  $n = 25$ , Fig. 4). This agreement indicates that our new ID-ICP-MS technique can produce comparable results with the conventional titration technique. However, a small difference in absolute values of  $\text{Ca}^{2+}$  concentration was detected. The term  $\Delta[\text{Ca}^{2+}]$  was defined in this study for assessing the difference in  $\text{Ca}^{2+}$  between the two methods:

$$\Delta[\text{Ca}^{2+}] = ([\text{Ca}^{2+}]_{\text{EGTA}} - [\text{Ca}^{2+}]_{\text{ID-ICP-MS}}) / [\text{Ca}^{2+}]_{\text{ID-ICP-MS}}, \% \quad (4)$$

In most cases, a lower  $\text{Ca}^{2+}$  was observed in the ID-ICP-MS data, compared with the titrations,  $\Delta[\text{Ca}^{2+}]$  ranged between

−1.05% and 3.77% with a median value of 1.63%. This offset is apparently much higher than the typical precision (0.1%) of the two analytical approaches.

There are two factors that may potentially affect the  $\Delta[\text{Ca}^{2+}]$  values. The heterogeneity of IAPSO seawater standard due to production of different batches may cause minor discrepancy in seawater  $\text{Ca}^{2+}$  quantifications, such as IAPSO utilized as the calibration standards in titration and ID-ICP-MS. This may be attributed to the small offset observed. On the other hand, as noted in previous studies,<sup>12,16,17</sup> the key issue for the accuracy of  $\text{Ca}^{2+}$  EGTA titration in seawater is the potential interference by  $\text{Mg}^{2+}$  and  $\text{Sr}^{2+}$ . Though seawater  $\text{Ca}^{2+}$  can be selectively titrated in the presence of  $\text{Mg}^{2+}$  owing to the sufficiently different stability constants between the complexes of Ca-EGTA ( $\log K_{\text{Ca-EGTA}} = 11.0$ ) and Mg-EGTA ( $\log K_{\text{Mg-EGTA}} = 5.2$ ), the presence of  $\text{Mg}^{2+}$  was found to produce certain errors on  $\text{Ca}^{2+}$  titration.<sup>12</sup> In particular, the contribution of interference from  $\text{Sr}^{2+}$  ( $\log K_{\text{Sr-EGTA}} = 8.5$ <sup>31</sup>) to the  $\text{Ca}^{2+}$  titration was reported, because  $\text{Sr}^{2+}$  was almost entirely titrated at the same time as  $\text{Ca}^{2+}$ .<sup>17</sup> A systematic investigation of seawater  $\text{Sr}^{2+}$  and Sr/Ca exhibits a spatial variation of 2–3% globally and represents a significant gradient in the vertical water column.<sup>32</sup> It implies potential inherent uncertainties associated with the traditional  $\text{Ca}^{2+}$  titration method. Two conventional solutions have been tried to solve this problem: (1) assume similar  $\text{Mg}^{2+}$  and  $\text{Sr}^{2+}$  present between the samples and adopted reference materials to eliminate the interference effect,<sup>15</sup> and (2) assume constant Mg/Ca and Sr/Ca ratios in seawater for potential artifact correction.<sup>12,17</sup> However, this assumption is valid only if similar contents of  $\text{Mg}^{2+}$  and  $\text{Sr}^{2+}$  present in all of the samples with adopted reference standards and the titrated Mg/Ca and Sr/Ca ratios meet the assumed values.

To further examine any systematic biases resulting in the noticeable  $\Delta[\text{Ca}^{2+}]$  between ID-MS and the titration method, high precision seawater metal/Ca ratios, including Mg/Ca and Sr/Ca, were determined using SF-ICP-MS based on a modified procedure from our ID-ICP-MS protocol. IAPSO seawater was also selected as a reference standard for metal/Ca determination with an average external precision (RSD) of 0.15%. The concentrations of  $\text{Mg}^{2+}$  and  $\text{Sr}^{2+}$  in seawater samples were estimated based on the measured metal/Ca ratios and

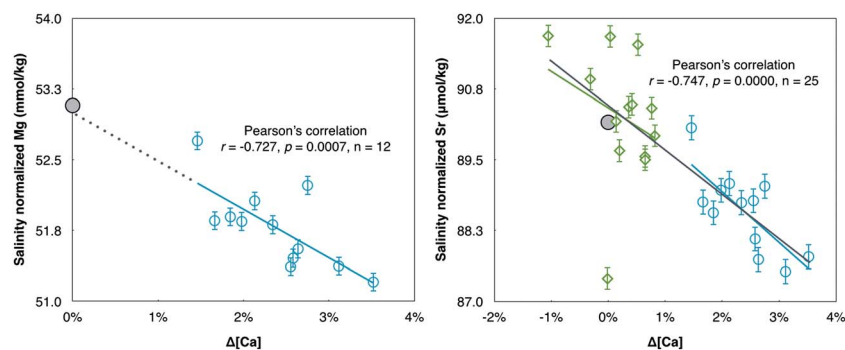


Fig. 5 The negative correlation between  $\Delta[\text{Ca}]$  and the presence of Mg (left) and Sr (right) in seawater, where Mg and Sr concentrations were normalized to salinity equalling to 35 psu. The black points represent the Mg and Sr contents of the IAPSO seawater standard,<sup>35</sup> and plotted at  $\Delta[\text{Ca}] = 0\%$ .

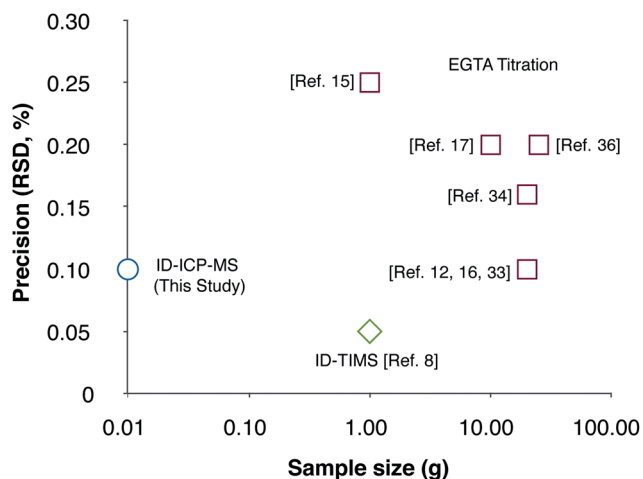


Fig. 6 Analytical precision and minimum sample requirement in seawater Ca determination using various EGTA titrations (squares), ID-TIMS (diamonds) and ID-ICP-MS (points).

corresponding high precision  $\text{Ca}^{2+}$  ID-ICP-MS data with a combined uncertainty of better than 0.20%. The results of metal/Ca ratios, seawater  $\text{Mg}^{2+}$  and  $\text{Sr}^{2+}$  coupled with salinity data, are shown in Fig. 3a and c–h. Based on our results,  $\text{Mg}^{2+}$  and  $\text{Sr}^{2+}$  data present a  $\text{Ca}^{2+}$ -like distribution in the vertical water column (Fig. 3e and f). It is noteworthy that  $\text{Mg}^{2+}$  and  $\text{Sr}^{2+}$  displayed non-conservative behavior related to the salinity and revealed an excess of metals in the shallow water zone and intermediate water, particularly at depths of around 250 m and 750 m (Fig. 3g and h). Furthermore, a significantly higher peak in the Sr/Ca ratio was also detected in the corresponding water mass (Fig. 3c). This  $\text{Sr}^{2+}$  enrichment and the higher Sr/Ca ratios imply the addition of sources of  $\text{Sr}^{2+}$  to the shallow water.<sup>32</sup> The variation in seawater Sr/Ca ratios is, therefore, a potential indicator reflecting the mixing of different water masses. The seawater Mg/Ca anomaly was also detected in the vertical water column (Fig. 3d). However, the resolution of seawater Mg/Ca ratios is insufficient to be a good water mass indicator under the consideration of analytical precision, potentially owing to  $\text{Mg}^{2+}$  being a major component in seawater chemical compositions. The classic Pearson's correlation was computed to evaluate the correlation between the  $\Delta[\text{Ca}^{2+}]$  and the

normalized  $\text{Mg}^{2+}$  and  $\text{Sr}^{2+}$ . The contribution of interference from  $\text{Mg}^{2+}$  ( $r = -0.727$ ,  $p = 0.0007$ ,  $n = 12$ ) and  $\text{Sr}^{2+}$  ( $r = -0.747$ ,  $p = 0.000$ ,  $n = 25$ ) to seawater  $\text{Ca}^{2+}$  titration was evidenced (Fig. 5). Besides, the magnitude of  $\Delta[\text{Ca}^{2+}]$  is strongly associated with the content of  $\text{Mg}^{2+}$  and  $\text{Sr}^{2+}$  in seawater compared with these two components in adopted reference standards, e.g. IAPSO seawater standard in this study. It further emphasizes that Mg/Ca and Sr/Ca variations in seawater certainly can bias the accuracy of  $\text{Ca}^{2+}$  EGTA titration and may cause detectable systematic uncertainty. It is noteworthy that a small but detectable variation in seawater Sr/Ca ratios provides critical information on mechanisms that control the  $\text{Ca}^{2+}$  and  $\text{Sr}^{2+}$  budgets in the surface water.<sup>32</sup> The assumptions used in the conventional titration techniques certainly omit these important signals. The new  $\text{Ca}^{2+}$  ID ICP-MS can therefore be an alternative method in oceanography and aquatic geochemistry, although the discrepancy to EGTA will continue to be discussed in the near future.

### Comparison with available techniques

A comparison of minimum sample size requirement, analytical time for each individual measurement, and methodological reproducibility using available EGTA titration, ID-TIMS and our new ID-ICP-MS techniques is summarized in Table 4 and Fig. 6. ID-MS provides the highest analytical precision with a small sample size of 1 g. Based on considerations of the analytical precision, ID-TIMS is the best choice for seawater  $\text{Ca}^{2+}$  determination. However, the disadvantages of ID-TIMS include the long time periods consumed by ion-chromatography purification, sample preparation, and analytical time, as well as its considerable experimental cost. EGTA titration generally has an analytical precision of 0.1% RSD, but its good precision relies on a large volume of 10 to 20 mL seawater per titration. It is clear that the analytical precision of the titration method is limited by the sample size and some basic assumptions about  $\text{Sr}^{2+}$  and  $\text{Mg}^{2+}$  interference. Our newly developed ID-ICP-MS method provides simple experimental and instrumental operation, efficient sample preparation, high sample throughput on a scale of less than 5 min, extremely minimal consumption, and an equally high precision of 0.1% RSD compared with the EGTA method.

Table 4 Technique evaluation of various methodologies for seawater Ca determination

Reference	Methodology	Minimum sample requirement (g)	Precision (RSD, %)	Time for single determination (min)
Tsunogai <i>et al.</i> (1968) <sup>12</sup>	EGTA titration	20	0.1	5
Tao <i>et al.</i> (2009) <sup>15</sup>		1	0.25	
Lebel and Poisson (1976) <sup>17</sup>		10	0.2	
Kanamori and Ikegami (1980) <sup>33</sup>		20	0.1	
Olson and Chen (1982) <sup>16</sup>		20	0.1	
Krumgalz and Holzer (1980) <sup>36</sup>		25	0.2	
Anderson and Grané (1982) <sup>34</sup>		20	0.16	
Ringbom <i>et al.</i> (1958) <sup>14</sup>	ID-TIMS	20	0.05	>60 (estimated)
de Villiers <i>et al.</i> (1998) <sup>8</sup>		1		
This study		ID-ICP-MS		



## 4. Conclusion

In this study, we describe an accurate method for precise determination of Ca in seawater by  $^{44}\text{Ca}/^{43}\text{Ca}$  ID-ICP-MS. An analytical precision of better than 0.05% (RSD) for within-run and 0.1% (RSD) for duplicate measurements of the seawater standard (IAPSO) was achieved using 10  $\mu\text{L}$  aqueous solutions in less than 3 minutes of measuring time. In addition, this technique is free from any potential spectral interference and low blanks, potentially making ID-ICP-MS a highly useful direct approach to precisely analyze  $\text{Ca}^{2+}$  in seawater. This method benefits markedly from the improved operational difficulties and enhanced analytical efficiency for precise  $\text{Ca}^{2+}$  determination in seawater compared with conventional titration and ID-TIMS techniques. The reliability of our new methodology was confirmed by its similarly accurate and precise results compared with well-developed EGTA approaches. Furthermore, the exquisite sensitivity of seawater Ca measurement by ID-ICP-MS provides important new information on  $\text{Ca}^{2+}$  sources in the upper ocean column and creates new opportunities to explore various applications in chemical oceanography.

## Acknowledgements

This work was supported by grants from NSC, Taiwan to CFY. We are grateful for critical comments and suggestions provided by Professor Dai on the earlier draft.

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