A High Precision Method for Calcium Determination in Seawater Using Ion Chromatography

Hangqi He, Yifan Li, Shuyuan Wang, Qianwei Ma and Yiwen Pan*

Department of Marine Science, Zhejiang University, Zhoushan, China

Calcium (Ca^{2+}) is an important major cation in seawater, which is closely related to the oceanic biogeochemistry cycle. Direct and accurate determination of Ca^{2+} concentration is required for a more comprehensive study of the carbonate system in seawater. Due to the high background concentration of Ca^{2+} in seawater and small variances of Ca^{2+} during $CaCO_3$ precipitation and dissolution process, a precision of better than 0.1% (of approximately $\pm$ 10 μmol/kg) is very much desired for carbonate chemistry related studies. In this study, a simple, non-toxic and labor-saving technique using ion chromatography (IC) has been developed to determine Ca^{2+} in seawater with an overall precision of better than 0.1%. Due to lack of available commercial calcium standard in seawater matrix, IAPSO seawater was selected as the reference after calibration. This proposed method can get a result within 15 min and only requires a small sample volume (~1 ml). The concentrations and flow rates of the eluent have been optimized to achieve the best chromatographic separation (20 mmol/L and 1.0 mL/min were selected in this study). Our work suggests that sample dilutions by weighing have no discernible effect on Ca^{2+} determinations. However, the measured Ca^{2+} concentration shows a linear decrease with the increasing Mg/Ca ratio in samples, which could be corrected by a derived formula to achieve high accuracy within 0.1%. This optimized method has been applied to the analysis of Ca^{2+} distribution in Southwest Indian Ocean and the laboratory study on the calcite precipitation in seawater.

Keywords: calcium concentration, seawater, ion chromatography, manual sample injection, high precision, Mg/Ca ratio

INTRODUCTION

Calcium (Ca^{2+}) is one of the major cations in seawater. Although Ca^{2+} concentration ($[Ca^{2+}]$) in open ocean waters is largely considered as a conservative value with respect to salinity (approximately 10.28 mmol/kg with a salinity of 35), processes including bio- and abio- genic precipitation, carbonate sediment dissolution etc., contribute to the deviations of a constant calcium/salinity ratio.

Calcium is the main component of aragonite and calcite ($CaCO_3$). The precipitation and dissolution of calcium carbonate is an important component of the oceanic carbon cycle. Recent estimates of both $CaCO_3$ production and export at a global ocean scale (~175×10^{12} mol/year) are roughly at the same order, suggesting relatively high carbon export efficiency in the form

of inorganic carbon (Smith and Mackenzie, 2016). The acidification effects caused by growth in atmospheric CO_2 have certainly reduced the degree of seawater $CaCO_3$ saturation state (Ω) and thus potentially reduce marine organisms' ability to form calcareous shells (Feely and Chen, 1982; Kleypas et al., 2001; Doney et al., 2009), and have increased the dissolution rate of dead biogenic remains (Andersson and Gledhill, 2013). When 1 mole of $CaCO_3$ precipitates or dissolves, the corresponding variations of Ca^{2+}, dissolved inorganic carbon (DIC) and total alkalinity (TAlk) are 1, 1, and 2 moles, respectively. Therefore, Ca^{2+}, DIC and TAlk could be used to study $CaCO_3$ precipitation/dissolution if a certain detection precision could be reached.

High quality DIC and TAlk data are provided by abundant studies, which form the knowledge base of present estimates of oceanic the $CaCO_3$ flux (Chung et al., 2003; Feely et al., 2004; Barrett et al., 2014; Smith and Mackenzie, 2016). However, reports of Ca^{2+} distribution are less popular than reports of DIC and TAlk. Besides, the contribution of dissolved organic matter (DOM) to seawater TAlk has been demonstrated in numerous studies (Chung et al., 2003; Feely et al., 2004; Cao and Dai, 2011; Barrett et al., 2014). The nutrients released during DOM decomposition will contribute a potential impact to the titration of seawater TAlk, which indicates the potential estimation bias of the $CaCO_3$ budget using observed TAlk and DIC data. Unlike DIC or TAlk, which is affected by both organic and inorganic carbon production and consumption, variations of Ca^{2+} are almost exclusively controlled by the $CaCO_3$ precipitation and dissolution when the influence of hydrothermal inputs is ignored. Due to $CaCO_3$ precipitation and dissolution process, the observed low ratios of calcium/salinity in surface waters and elevated ratios in deep waters have been recognized for a long time in the oceans (Riley and Tongudai, 1967; Chen, 1978; Krumgalz, 1982), and the variation of $[Ca^{2+}]$ is ~1% of the total amount in seawater. Thus, accurate quantification of $[Ca^{2+}]$ is necessary for looking into the marine $CaCO_3$ cycle and the advantage is also obvious.

For any analysis method, precision is the degree of proximity of multiple measured values for the same sample, which is an inherent quality of the method itself. Accuracy is the degree of conformity between the measured value and the real value, which is mainly related to the standard used. A high precision is the prerequisite of high accuracy. In the analysis of DIC and TAlk, a precision of better than 0.1% (of approximately $\pm$ 2 μmol/kg) could be routinely obtained (Sabine et al., 2002; Dickson et al., 2007; Cai et al., 2011). When using the certified Dickson seawater as the reference, the DIC and Talk analysis can achieve a high accuracy within 0.1%. Due to the high background concentration of Ca^{2+} in seawater and small variances of Ca^{2+} during $CaCO_3$ precipitation/dissolution process, a precision of better than 0.02% (of approximately $\pm$ 2 μmol/kg) for Ca^{2+} analysis is required as a similar data precision level of DIC and TAlk, which is not realistic at this stage. Considering the limitation, precision of at least better than 0.1% (of approximately $\pm$ 10 μmol/kg) is very much desired for carbonate chemistry related studies (Olson and Chen, 1982; Milliman et al., 1999; Jiang et al., 2010; Millero, 2013). Thus, achieving the

required precision ($<$0.1%) is an important basis for the accurate Ca^{2+} analysis. Besides, the lack of commercial calcium standard in seawater matrix is a obstacle for calcium determination in seawater at present.

Over the past decades, complexometric titration has been developed to determine Ca^{2+} in seawater with a high precision. Ca^{2+} can be selectively titrated with ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) in the presence of Mg^{2+} (Schmid and Reilley, 1957; Culkin and Cox, 1966; Tsunogai et al., 1968). Ion selective electrodes are incorporated as end-point indicators for achieving a good precision (Whitfield and Leyendekkers, 1969; Hadjiioannou and Papastathopoulous, 1970; Lebel and Poisson, 1976; Kanamori and Ikegami, 1980). Corrections of Mg^{2+} and Sr^{2+} interferences are also required to get an accuracy of better than 0.1% (Grasshoff et al., 2009). Cao and Dai (2011) modify the classic EGTA titration from Lebel and Poisson (1976) with an amalgamated silver electrode for end-point detection, which achieves a better precision of 0.06%. Despite the high precision of this method, a large amount of toxic mercuric chloride must be added into the sample before titration, which is not friendly to the operator and the environment. Besides, there are some common problems in these titration methods. For example, these methods are laborious and time consuming, meanwhile, a large sample volume (~4 to 25 ml) is always required for a single measurement.

In recent years, ion chromatography (IC) has been developed rapidly. It has many advantages over other analytical methods in speed, selectivity, stability and simultaneous determination ability. The principle of IC is based on the reversible exchange between dissociable ions on the ion exchange column and solute ions with the same charge in the mobile phase. As a result, different components with different affinities are separated by the ion exchanger. Therefore, IC can be applied to the simultaneous separation and determination of common cations in aqueous solutions. There are many reports about the studies of Ca^{2+} determination using IC (Thomas et al., 2002; Garcıá-Fernández et al., 2004; Zeng et al., 2006), but the applications are restrained in samples of low ionic strength matrix and low Ca^{2+} concentration, such as mineral waters and drinking water. A few studies have reported that both Mg^{2+} and Ca^{2+} can be simultaneously detected in marine sediment porewaters (Melendez et al., 2013) and samples of high ionic strength matrix (Laikhtman et al., 1998) but with a low precision (1–2%). Thus, the challenge in applying the IC method in complex matrix samples and the low precision are two key factors that restrain its application in accurate Ca^{2+} determination in seawater.

In this study, we have described the implementation of an IC method for determining Ca^{2+} with required precision ($<$0.1%) in seawater. The main advantages of this method include all the benefits of IC method: a small sample volume (~1 ml of seawater), elimination of complex sample interferences, and simple analyzing operation. Considering there is no available commercial calcium standard in seawater matrix, the seawater reference from International Association for the Physical Sciences of the Oceans (IAPSO) was selected as the reference for Ca^{2+} determination and a high accuracy can be achieved.

The effects of sample dilutions, Mg^{2+} and Sr^{2+} on the Ca^{2+} determinations are investigated as well.

EXPERIMENT

Apparatus

A Dionex model ICS-5000 + ion chromatograph (Thermo Fisher) was employed along with a Chromeleon chromatography workstation for instrument control as well as data acquisition and processing in conductivity detection. A Dionex Ion Pac CG 12A (4 mm × 50 mm i.d.) guard column and Dionex Ion Pac CS 12A (4 mm × 250 mm i.d.) separation column were used. The equipped sample loop was 100 μL and the eluent was 20 mM Methanesulfonic acid (MSA). In cation chromatography, the ion exchange functional group of resin filled with separation column was sulfonic acid ($-SO_3^-$), and the corresponding eluent was methanesulfonic acid (MSA). After separation, the corresponding signal was obtained by the conductometric method. A Dionex CERS 500 (4 mm) cation self-regenerating suppressor was applied in the system to reduce the background conductivity and thus improved the detection limit. Column oven was set at 30°C for all separations and the conductivity cell was kept at a constant 35°C.

Reagents and Standards

Preparation of artificial seawater with known $[Ca^{2+}]$ is a feasible method to obtain the calcium standard, but it will greatly increase the workload and there may be some potential errors in the preparation process due to the different operators. Although IAPSO seawater is a salinity standard, the $[Ca^{2+}]$ of each individual IAPSO batch is stable. Thus, IAPSO seawater of the same batch can be considered as a stable calcium reference. According to the stoichiometric reference composition of the standard seawater defined by Millero et al. (2008), the average $[Ca^{2+}]$ calculated in seawater is 10.28 mmol/kg at a salinity of 35 and the corresponding Ca^{2+}/Sal ratio is 293.7. Due to the preparation on the basis of Atlantic Ocean surface seawater, there is some variations of $[Ca^{2+}]$ in different batches of the IAPSO seawater. Several studies has reporterd the Ca^{2+}/Sal ratio in IAPSO seawater, such as Batch P61 [291.3 (Shiller and Gieskes, 1980)], Batch P79 [290.5 [(Olson and Chen, 1982)], and Batch P147 [292.3 (Cao and Dai, 2011)]. The Ca^{2+}–Sal ratios of different batches show a range of as much as 0.3–1%, which is well outside the desired accuracy. Therefore, the calibration of IAPSO seawater is necessary. After calibration by a known Ca^{2+} standard or the other measurement methods (such as complexometric titration method), IAPSO seawater of the same batch can be used as the calcium standard. However, this means that every lab would have to resort to calibrating their own batch of IAPSO seawater using a secondary method that they might not be familiar with. Besides, the accuracy of this method essentially relies entirely on the calibration result, which may be the main limitation of this method.
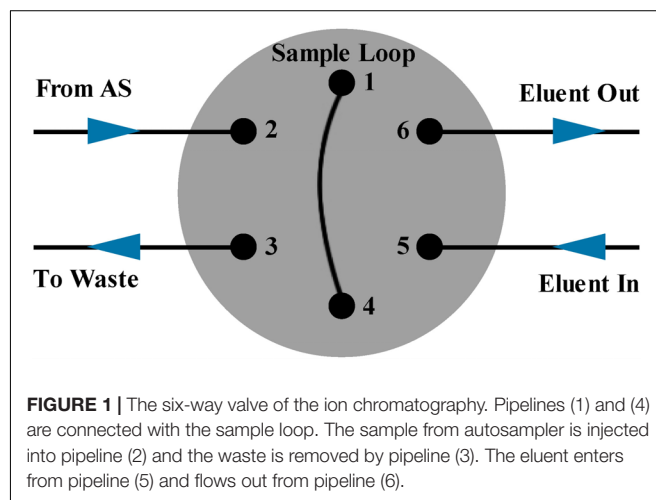
In this study, the Batch P162 of IAPSO seawater (salinity = 34.993) was selected as the calcium standard. An artificial seawater with known Ca^{2+} concentrations (by weighing) was prepared to calibrate this batch of IAPSO seawater. The $[Ca^{2+}]$ measured was 10.168 mmol/kg and the Ca^{2+}/Sal ratio was determined to be 290.6. Eluents were prepared by using extra pure grade Methanesulfonic acid (Acros Organics, 99%). The calcium, magnesium and strontium standard stock solutions (1000 mg/L) were bought from the Chinese National Standard Material Center (the corresponding product numbers were BW2021-1000-W-50, BW-2020-1000-W-50, and BW30023-1000-C-50), which were diluted to the designed concentrations and used as standards in recovery test. The deionized water (resistivity 18.2 MΩ·cm) used in both preparations of eluent and dilution of seawater samples was obtained from the Milli-Q water purification system (Millipore).

Procedures

In order to avoid overloading of the column caused by excess sodium ions in solutions, standards and seawater samples were diluted by weight with a suitable dilution factor according to the estimated $[Ca^{2+}]$. After dilution, the highest peak shape of Na^+ in seawater sample was not distorted, thus the peaks of Mg^{2+} and Ca^{2+} could keep their normal shapes. Considering the accuracy of the analytical balance (0.1 mg), 1 g of seawater sample or standard was weighed for dilution, which could reduce the error caused by weighing.

In order to achieve high precision, the precision of detecting part and autosampler part was tested separately. According to the test result, the uncertainty of the autosampler injection volume was of approximately 0.3%, which exceeded the required precision (<0.1%), thus the manual sample injection was used instead of autosampler injection. The connection mode of six-way valve pipeline was shown in **Figure 1**. The injection pipeline (2) of the six-way valve was directly connected to the sample solution, and a syringe was connected to the waste pipeline (3). Before injection, the syringe was pulled to form a vacuum and ~2 mL diluted sample solution was thus sucked through the sample loop due to the pressure differences, that guaranteed the sample loop was cleaned and completely filled. Large volume

FIGURE 1 | The six-way valve of the ion chromatography. Pipelines (1) and (4) are connected with the sample loop. The sample from autosampler is injected into pipeline (2) and the waste is removed by pipeline (3). The eluent enters from pipeline (5) and flows out from pipeline (6).

injection was chosen by using big sample loop (100 µL) to reduce the error caused by manual injection.

In previous IC methods, Mg^{2+} and Ca^{2+} could be separated and detected from excess sodium solution by sample solution complexation (Garciá-Fernández et al., 2004; Melendez et al., 2013) or double pump separation system of IC (Laikhtman et al., 1998). The complex sample pretreatment and separation process might cause the loss of the detected Ca^{2+}, reducing the precision of the analysis method. Therefore, in this study, we tried to simplify the sample pretreatment and subsequent analysis process to eliminate potential errors. There was no complex sample pretreatment except the necessary dilution, the injection and separation pipelines were also minimized in the IC analysis process. Compared with the previous methods, the precision of this method has been significantly improved.

RESULTS AND DISCUSSION

Precision and Reproducibility

The precision of the method for Ca^{2+} in seawater was investigated by analyzing five runs of a sample. To compare the precision of autosampler injection method and manual injection method, reproducibility was tested using the diluted Dickson reference seawater (Batch 154) and the single calcium standard solution by manual injection and automatic injection, respectively. The retention time between these two different injection methods shows a similar pattern while the relative standard deviation (RSD) for the Ca^{2+} peak area exhibits some differences. The RSD is 0.05% in manual injection method while 0.45% in automatic injection method ($n = 5$) (**Table 1**). Only the manual injection method fulfills the <0.1% requirement of Ca^{2+} analysis in seawater. The very stable retention time in both automatic injection method and manual injection method (RSD = 0.04%) implies that the reproducibility in ion-exchange separation process (Mg^{2+}, Ca^{2+}, and Sr^{2+}) is very stable. The differences in peak area reproducibility between the automatic injection and manual injection method in both seawater sample and the single calcium standard solution test indicates that it is the reproducibility in automatic injection method that limits the whole precision of IC analysis.

The sample injection volume should be controlled by the sample loop (100 µL) in principle. In the process of automatic injection, the sample solution was extracted into a syringe-type

pump from the sample bottle through the pump and the needle moved to the injection pipeline (2) in **Figure 1** to push the sample solution into the sample loop. During samples extraction and injection, small bubbles from the atmosphere might be introduced into the pipeline and stayed in the sample loop. When switching to manual injection method, a syringe-type pump, a six-way valve and some loops before sample entering through the other six-way valve (**Figure 1**) into the sample loop (100 µL) which were included in automatic injection method were abandoned. Instead a syringe and a short loop were connected to a six-way valve directly, and the pipeline (2) in **Figure 1** was always in the sample bottle to avoid the introduction of bubbles. On the other hand, the automatic injection mode is to "push" samples into the sample loop. This mode might slightly compress the solution and small bubbles were more likely to stay in the sample loop, which would increase potential variations in the sampling volume. While the manual injection mode is to "suck" samples into the sample loop by the pressure differences, the sample loop could be filled uniformly by the sample solution and it was not easy to form bubbles. By manual injection, the sample loop could stably control the injection volume to improve the whole precision of IC analysis. Therefore, the manual injection method exhibited a much higher reproducibility than autosampler method.

Optimization of the Chromatographic Separation

The basic principle of IC is to measure ionic species' concentrations after separating them based on their interactions with a resin column. Before detection, sample solutions pass through a pressurized chromatographic column. After first absorbing by column constituents and separating by eluents of the ions, various ions in a sample could be separated due to their different retention time. Thus, the retention time of different species not only depends on species type, size, column materials, but also is influenced by the eluent type, the concentration and the flowing rate. For seawater sample (**Figure 2**), which is a complex matrix medium, Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, and Sr^{2+} could be separated and detected in turn. The retention time of Mg^{2+}, Ca^{2+}, and Sr^{2+} are close to each other due to their similar ionic properties. In order to achieve a good separation effect of Mg^{2+}, Ca^{2+}, and Sr^{2+} in seawater, different eluent concentrations and flow rates, as well as dilution effects on samples were tested. Take the separation

TABLE 1 | Results of precision analysis for Ca^{2+} concentration in Dickson reference seawater (Batch 154, salinity = 33.347) and the single calcium standard solution using manual injection and automatic injection, respectively.

	Injection method	Dilution factor	Retention time (min)	RSD% (n = 5)	Peak area (µs·min)	[Ca²⁺] (mmol/kg)	RSD% (n = 5)
Dickson reference seawater	Manual injection	1:50.6	10.282 ± 0.004	0.04%	8.690 ± 0.004	9.812 ± 0.005	0.05%
	Automatic injection	1:50.6	10.214 ± 0.004	0.04%	8.61 ± 0.04	9.80 ± 0.04	0.45%
Calcium standard solution	Manual injection	1:128.8	10.289 ± 0.004	0.04%	8.822 ± 0.006	24.95 ± 0.02	0.07%
	Automatic injection	1:125.8	10.471 ± 0.006	0.06%	9.04 ± 0.02	24.95 ± 0.06	0.26%
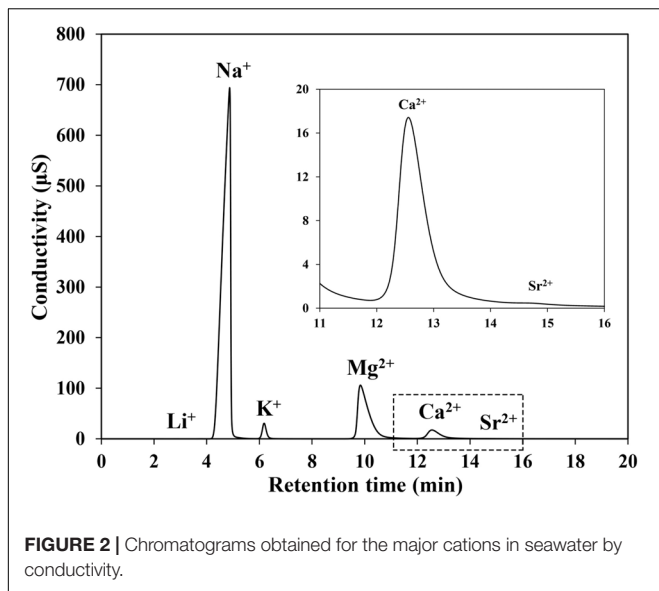
FIGURE 2 | Chromatograms obtained for the major cations in seawater by conductivity.

TABLE 2 | The resolution of Mg^{2+} and Ca^{2+} in seawater with different eluent concentrations and flow rates.

Concentration (mmol/L)	Flow rate (mL/min)	Peak area of Ca^{2+} ($\mu s \cdot min$)	Retention time of Ca^{2+} (min)	Resolution
15	0.8	10.670	20.963	3.516
	1.0	8.532	16.833	3.394
	1.2	7.100	14.073	3.279
20	0.8	10.577	13.513	3.183
	1.0	8.449	10.847	3.015
	1.2	6.994	9.070	2.881
25	0.8	10.328	9.930	2.752
	1.0	8.223	7.977	2.590
	1.2	6.822	6.660	2.453

degree between Mg^{2+} and Ca^{2+} as an example, it can be described by the resolution of Mg^{2+} and Ca^{2+}, which is expressed as:

$$R = \frac{2(t_R\,(Ca) - t_R\,(Mg))}{W\,(Ca) + W\,(Mg)} \tag{1}$$

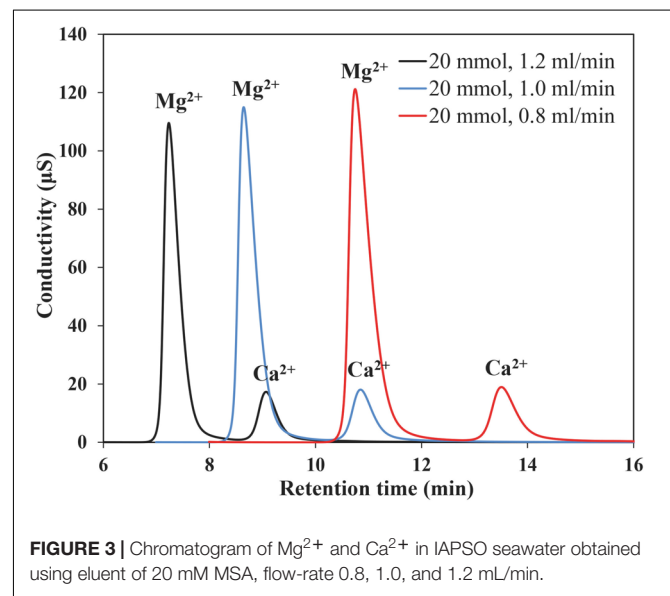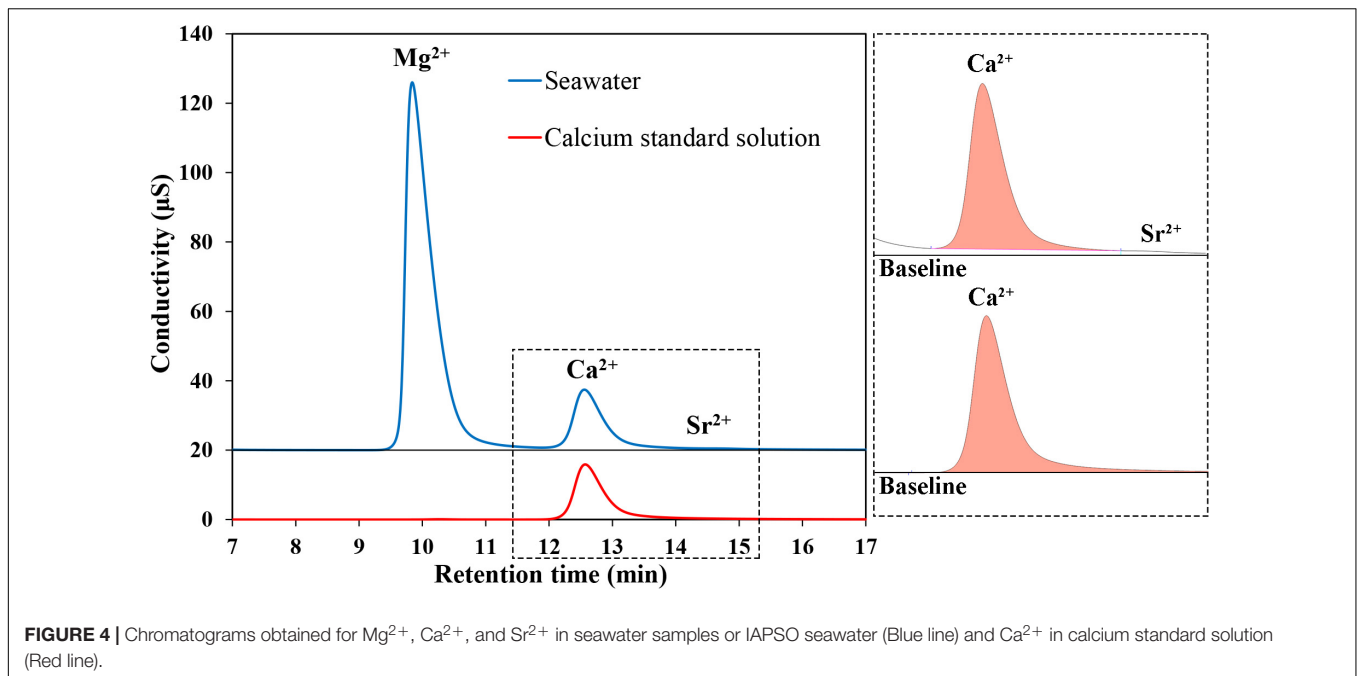where R is the resolution, t_R is the retention time and W is the peak width.

The separation degree (R) under three different eluents' concentrations and flow rates are listed in **Table 2**. No significant changes in retention or peak shapes were observed under all rinsing conditions (**Figure 3**), although cation separation degree, retention time and peak area of Mg^{2+} and Ca^{2+} were found to decrease with enhanced eluent concentrations and flow rates. In the requirement of routine IC analysis, ions can be considered to be totally separated if R is greater than 1.5. Considering the retention time and resolution, the eluent concentration of 20 mmol/L and the flow rate of 1.0 mL/min are selected in the present study. In this condition, Mg^{2+} and Ca^{2+} in seawater can be well separated in less than 15 min (**Figure 3**), which makes the whole measurement process time-saving.

Reference Solution

Due to the similar ionic properties among Mg^{2+}, Ca^{2+}, and Sr^{2+}, the retention times of Mg^{2+}, Ca^{2+}, and Sr^{2+} are close to each other (**Figure 2**). In seawater, the concentration of Mg^{2+} is nearly four times higher than Ca^{2+}, while Sr^{2+} is 100 times lower. Although good separation effects between Mg^{2+} and Ca^{2+} ($R > 2.5$) could be achieved with the eluent concentration and flow rate of 20 mmol/L and 1.0 mL/min, there are small tailing peaks of Mg^{2+} which cause partial overlap between Mg^{2+} and Ca^{2+} peaks, albeit only to a small degree (**Figure 4**). Instead, the peak area of Sr^{2+} is very small after separated by the IC column (**Figure 2**) but still has a partial overlap with Ca^{2+} peak. Due to the lower concentration compared with Mg^{2+}, Sr^{2+} has

FIGURE 3 | Chromatogram of Mg^{2+} and Ca^{2+} in IAPSO seawater obtained using eluent of 20 mM MSA, flow-rate 0.8, 1.0, and 1.2 mL/min.

less influence on accurately determining Ca^{2+} in seawater-like matrix sample by IC method. The partial overlap between Mg^{2+}, Ca^{2+}, and Sr^{2+} peaks might impair the accuracy of IC analysis, but it is hard to eliminate this phenomenon completely only by changing the separation conditions of IC. Therefore, IAPSO seawater was selected as the reference in this study, the partial overlap phenomenon existing in both standard and sample could eliminate its influence on the analysis results.

Take the Dickson seawater (Batch 154, salinity = 33.347) as a sample, the $[Ca^{2+}]$ measured using IAPSO seawater and the single calcium standard solution as references were 9.812 mmol/kg and 9.697 mmol/kg, respectively. Differences (~1%) in the measured $[Ca^{2+}]$ using different reference solutions could be observed. Comparing the Ca^{2+} peak shapes and integration areas of seawater and zero salinity Ca^{2+} standard (the single calcium standard solution) (**Figure 4**), small differences in the baseline between the two Ca^{2+} peak shapes could be observed. It is the tailing of Mg^{2+} peak that causes a little overlap with the Ca^{2+} peak. When using the single calcium standard solution as the reference, there is no tailing effect of Mg^{2+} peak.

FIGURE 4 | Chromatograms obtained for Mg^{2+}, Ca^{2+}, and Sr^{2+} in seawater samples or IAPSO seawater (Blue line) and Ca^{2+} in calcium standard solution (Red line).

The peak shapes of the reference and the sample differ with each other, which would cause discrepancy in the measured Ca^{2+} value. Thus, the results obtained are not extremely accurate and would be affected by the integration method, such as the selection of the integral interval. By comparison, the similar component between IAPSO seawater and the sample would form similar peak shapes, which could potentially eliminate the influence of Mg^{2+} and Sr^{2+}. And different integration methods would hardly affect the measured result. Therefore, IAPSO seawater is a better alternative than the single calcium standard solution as a reference when measuring seawater sample.

Dilution Effect and Standard Procedure

Samples like seawater need to be diluted before detecting using IC method, because high concentration of ions could casue column overloading problem without diluting. Effect of the dilution factor was tested by detecting the $[Ca^{2+}]$ in IAPSO seawater after being diluted by a factor of 25, 38, 50, 75, 100, 150 times by weight. A good linear relationship between the Ca^{2+} concentration and detected peak area ($R^2 = 0.9999$) was observed, which indicates good responses of the Ca^{2+} in the diluted seawater within the range of 0.07–0.43 mmol/kg. Since the concentration of Ca^{2+} is relatively stable in seawater, we narrow the concentration range of the analytical curve to 0.18–0.22 mmol/kg (i.e., diluted 40–60 times), and dilute the seawater samples approximately 50 times.

A negative intercept of the calibration curve between detected Ca^{2+} peak area and the $[Ca^{2+}]$ could be observed in **Figure 5**. If the peak area of Mg^{2+} and the Mg^{2+} concentration was fitted, good linear fitting could also be observed with a slightly positive intercept. The phenomenon corresponds to the hypothesis that the peak area of Ca^{2+} in seawater may be underestimated due to the overlap between the peaks of Mg^{2+} and Ca^{2+}, while the peak area of Mg^{2+} may be overestimated on the contrary. It should be

noted that the reduced peak area is closely related to the rinsing conditions and the integration method.

Accuracy and Interferences of Mg^{2+} and Sr^{2+}

The accuracy of any method can only be determined relative to a reference standard that has been certified by several independent laboratories, typically using a number of different techniques. However, there is no such certified seawater standard for Ca^{2+}. Therefore, in this study, the accuracy of this method was determined through the recovery test. Three calcium standards with certain concentrations were obtained by adding a certain amount of calcium standard solution to IAPSO seawater. According to the narrow variation range of $[Ca^{2+}]$ in seawater, the $[Ca^{2+}]$ of IAPSO seawater increased by 0.5, 2, and 5%, respectively, relative to the initial concentration. The density of the calcium standard solution was 1.000 g/cm^3 at 25°C determined by gravimetric method. As shown in **Table 3**, the recovery ratio is 100 ± 0.3%, which is slightly larger than the required accuracy (100 ± 0.1%). However, comparing with the recovery test results of other IC methods, the recovery ratio of the present method is much better than that of others, whose errors are approximately 1–5% (Thomas et al., 2002; Zeng et al., 2006; Caland et al., 2012; Melendez et al., 2013). It's worth noting that there may be some potential errors in the recovery test, such as the change of solution density and Mg/Ca ratio in standard preparation, the accurate value of $[Ca^{2+}]$ calculated after addition, etc. The error of recovery ratio (0.3%) is partly caused by the standard preparation. Thus, the recovery ratio cannot well represented the accuracy of this method, which may be better than 100 ± 0.3%. Nonetheless, until an independently Ca^{2+}-certified natural seawater reference
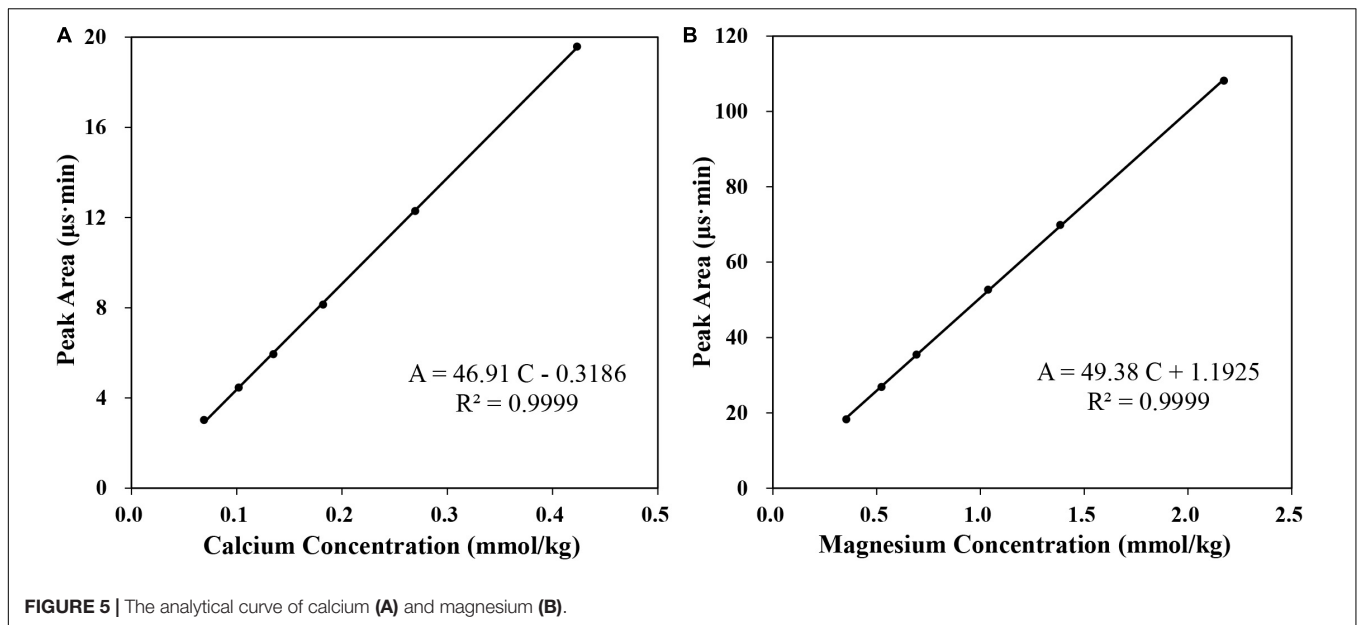
FIGURE 5 | The analytical curve of calcium **(A)** and magnesium **(B)**.

becomes widely available, the accuracy of this method remains to be further determined.

In order to study the effect of Mg^{2+} and Sr^{2+} concentrations on Ca^{2+} analysis, zero salinity Mg^{2+} and Sr^{2+} standards were separately added into IAPSO seawater to produce samples of Mg/Ca ratio from 1 to 2.01 and Sr/Ca ratio from 1 to 7.08 that of seawater ratio. In each case, Ca^{2+} was determined at least three times to ensure the results agreeing within 0.1%. Results of these analyses are presented in **Table 4**. With the increasing Mg^{2+} concentration, the measured $[Ca^{2+}]$ deviates from the real value and decreases with increasing of the Mg^{2+} concentration or Mg/Ca ratio in sample.

When the measured $[Ca^{2+}]$ is plotted against the Mg/Ca ratio in the sample (**Figure 6**), a good linearity could be obtained ($R^2 = 0.995$), which provides a method to correct the underestimate of $[Ca^{2+}]$ in the sample caused by deviated Mg/Ca ratios. The correction equation could be described as follows:

$$[Ca^{2+}]_{correcting} = [Ca^{2+}]_{measured} + (R_{sample} - R_{IAPSO}) \times |K| \tag{2}$$

where $[Ca^{2+}]_{correcting}$ is the Ca^{2+} concentration after correction; $[Ca^{2+}]_{measured}$ is the Ca^{2+} concentration measured by IC; R is the Mg/Ca ratio, which could be obtained from measured Mg^{2+} and Ca^{2+} concentrations; K is the slope of the regression fitting equation, which relates to the parameters including the eluent concentration, flow rate, etc. According to the linear fitting equation ($C = -0.01394 R + 10.25$) and the Mg/Ca ratio in IAPSO seawater (5.137), Eq. 2 could be rewritten as Eq. 3.

$$[Ca^{2+}]_{correcting} = [Ca^{2+}]_{measured} + (R_{sample} - 5.137) \times 0.01394 \tag{3}$$

After correction with Eq. 3, the Ca^{2+} concentrations of samples with different Mg/Ca ratios, listed in **Table 5**, show good accuracy with deviation within 0.1%. Sr^{2+} has a similar effect on the Ca^{2+} measurement and the results can also be corrected with a similar correction method. Considering that the Sr^{2+} concentration in seawater is extremely low and the variation of Sr/Ca ratio is only approximately 2–3% (Villiers, 1999), the influence of Sr^{2+} on the Ca^{2+} measurement can be neglected by IC method when using IAPSO seawater as a reference.
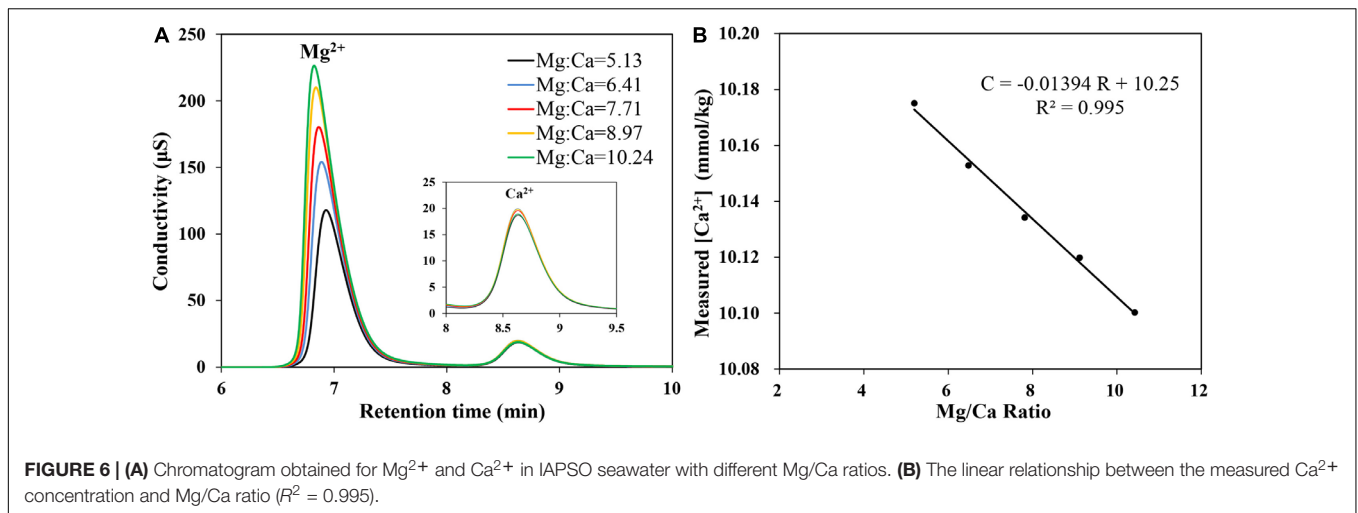
TABLE 3 | Results of recovery test by adding known calcium standard solution to IAPSO seawater.

Initial concentration (mmol/kg)	Ca^{2+} Added (mmol/kg)	Ca^{2+} Taken (mmol/kg)	Ca^{2+} Measured (mmol/kg)	Recovery ratio Measured/ Taken
10.168	0.000	10.168	10.169	100.00%
10.168	0.051	10.219	10.193	99.75%
10.168	0.201	10.369	10.366	99.98%
10.168	0.500	10.668	10.637	99.71%

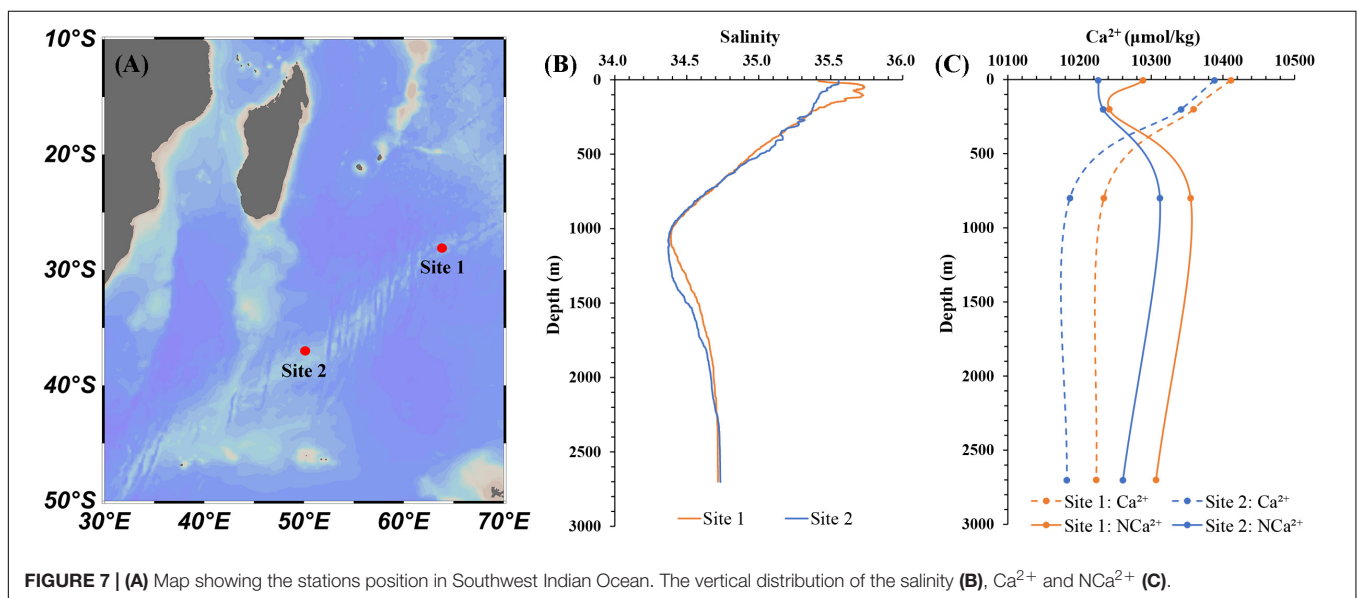**TABLE 4 |** Study of the effects of Mg^{2+} and Sr^{2+} on Ca^{2+} determinations.

Mg/Mg_{sw} Ratio	Sr/Sr_{sw} Ratio	Initial concentration (mmol/kg)	Measured (mmol/kg)	Ca Ratio Measured/ Initial
1	1	10.168	10.175	100.07%
1.25	1	10.168	10.153	99.85%
1.50	1	10.168	10.134	99.67%
1.76	1	10.168	10.120	99.53%
2.01	1	10.168	10.100	99.33%
1	3.07	10.168	10.045	98.79%
1	5.05	10.168	10.005	98.39%
1	7.08	10.168	9.940	97.76%

Mg/Mg_{sw} ratio and Sr/Sr_{sw} ratio in the table are relative to their seawater values.

FIGURE 6 | (A) Chromatogram obtained for Mg^{2+} and Ca^{2+} in IAPSO seawater with different Mg/Ca ratios. (B) The linear relationship between the measured Ca^{2+} concentration and Mg/Ca ratio (R^2 = 0.995).

TABLE 5 | Results of Ca^{2+} concentration after correction according to Mg/Ca ratio.

Mg/Ca Ratio measured	Initial concentration (mmol/kg)	Measured (mmol/kg)	Ca Ratio Measured/Initial	After correcting (mmol/kg)	Ca^{2+} Ratio Correction/Initial
5.13	10.168	10.175	100.07%	10.175	100.07%
6.41	10.168	10.153	99.85%	10.171	100.02%
7.71	10.168	10.134	99.67%	10.170	100.02%
8.97	10.168	10.120	99.53%	10.173	100.05%
10.24	10.168	10.100	99.33%	10.171	100.03%

FIGURE 7 | (A) Map showing the stations position in Southwest Indian Ocean. The vertical distribution of the salinity (B), Ca^{2+} and NCa^{2+} (C).

Application of This Method

The optimized Ca^{2+} determination method by IC has good sensitivity and reproducibility which enables precise analysis of small variations of $[Ca^{2+}]$ in high ionic strength seawater, that will contribute to a more comprehensive study of the carbonate system in seawater. We applied this method to analyze the Ca^{2+} distribution in natural seawater. As shown in **Figure 7A**, water samples were collected from two stations in Southwest Indian Ocean in November 2018. Water column samples were collected with Niskin bottles and stored in 100 mL acid-cleaned polyethylene bottles. Calcium were determined by this method and salinity were determined shipboard with the Conductivity-Temperature-Depth (CTD) recorder. **Figure 7B** shows the depth profiles of salinity at the sampling stations and **Figure 7C** shows the vertical distribution of the Ca^{2+} and normalized Ca^{2+} (NCa^{2+}) calculated by the Eq. 4. The $[Ca^{2+}]$

**TABLE 6 |** Results of the reacting solution components (three parallel groups).

	Ca^{2+} (μmol/kg)	DIC (μmol/kg)	TAlk (μmol/kg)	pH
Initial	10251 ± 1	2268 ± 1	2812 ± 1	8.521 ± 0.005
Final	9976 ± 17	2012 ± 5	2254 ± 5	8.091 ± 0.007
Δ	275 ± 18	256 ± 6	558 ± 6	0.430 ± 0.012

gradually decreases from 0 to 200 m and there is a rapid decrease up to 800 m, while at higher depths is found to remain constant, which is consistent with the investigation of Naik et al. (1983). The profiles of NCa^{2+} indicates that the ratios of Ca^{2+}/Sal in deep waters is higher than that in surface waters, which is the result of $CaCO_3$ precipitation and dissolution process. It is also noted that the NCa^{2+} of medium-deep waters in Site 2 is lower than that in Site 1. There are more accumulations of dissolved Ca^{2+} in Site 1 at this depth due to the "older" Antarctic intermediate water and North Atlantic deep water. The studies of Horibe et al. (1974) and Villiers (1998) found similar variations in the NCa^{2+} of deep water in different ocean basins. The high resolution of sampling is needed for a detailed discussion.

$$NCa^{2+} = \frac{[Ca^{2+}]}{\text{Salinity}} \times 35 \qquad (4)$$

Benefit from the small required sample volume and the simple measurement operation, this method can also be applied to the laboratory study of calcium carbonate precipitation and dissolution, but having little effect on the reaction solution volume. In this study, we studied the precipitation rate of calcite in seawater with this method. The calcite crystal growth experiments were conducted by adding calcite seeds (0.1 g) into the sealed Duran bottles. The calcite growth would initiate after adding 500 ml artificial seawater with initial saturation (Ω) of $\sim$12. The artificial seawater was prepared following Kester et al. (1967). During the experiments, 10 ml aliquots of the reacting solution were sampled every 2 to 3 days, and filtered through a 0.45 μm membrane for later analysis. pH was measured at $25 \pm 0.01°C$ with an Orion Ross combination glass pH electrode calibrated against three NIST standard buffers (4.01, 7.00, and 10.01). DIC was measured in triplicate using a DIC analyzer (Apollo Scitech, AS-C3). $[Ca^{2+}]$ was determined by this method. TAlk was detected by the Gran Titration (Millero et al., 1993) only for the initial and final reacting solution to save sample volume. Both DIC and TAlk were calibrated against the Dickson reference seawater and the precision was better than 0.1%. When calcite precipitated, the corresponding reduction of $[Ca^{2+}]$, DIC and TAlk should follow the theoretical ratio of 1: 1: 2. Comparing the variations of $[Ca^{2+}]$, DIC and TAlk parameters in solution before and after precipitation, the average ratio of ΔCa: ΔDIC: ΔTAlk is 0.986: 0.917: 2 (**Table 6**).

The ΔCa: ΔTAlk ratio of 0.986:2 deviates by about 1% from the theoretical ratio of 1:2, which is more than the combined precisions of the IC (Ca^{2+}) and titrations (TAlk) methods. Because when calculate the Δ, the absolute errors of $[Ca^{2+}]$
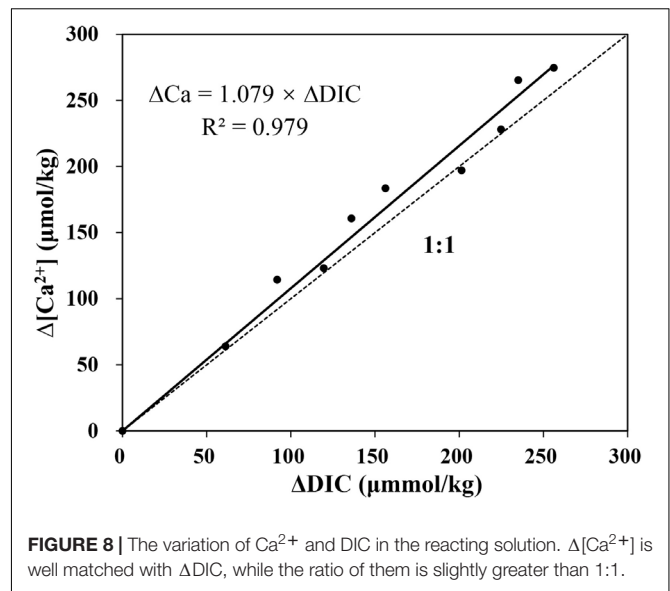
FIGURE 8 | The variation of Ca^{2+} and DIC in the reacting solution. $\Delta[Ca^{2+}]$ is well matched with ΔDIC, while the ratio of them is slightly greater than 1:1.

and TAlk are still preserved (even larger) as shown in **Table 6**. Besides, the errors of ΔCa and ΔTAlk are partly caused by three parallel experiments. Thus, considering these errors, the relative error of ΔCa and ΔTAlk will be more than 1%. If we assume that the measured value of ΔTAlk (558 ± 6 μmol/kg) was absolutely accurate, then the expected value of ΔCa would be 279 ± 3 μmol/kg. In fact, the measured value of ΔCa is 275 ± 18 μmol/kg, which is consistent with the expected value. Although the ΔCa: ΔTAlk ratio is as expected, due to the large absolute error (of approximately $\pm$ 10 μmol/kg) of calcium determination, there may be some deviation when establishing the relationship between $[Ca^{2+}]$ and TAlk in natural seawater. This also indicates that the current precision of calcium determination ($<$0.1%) is not enough, which needs to reach better than 0.02% as mentioned above.

Reduction in DIC shows a relatively smaller value in comparison. When the variations of $[Ca^{2+}]$ is depicted against DIC reduction at a regular interval, good linearity is achieved when the least squares linear fitting algorithm is used to fit the data. The slope of the regression is close to 1.08 (**Figure 8**). The deviation of slope from the theoretical value 1 may be caused by the DIC data, which could be partly influenced by atmospheric CO_2 during sampling. This result suggests that the optimized method for determination of $[Ca^{2+}]$ has a good response to the small variation of Ca^{2+} in seawater. More importantly, compared to DIC, which could be influenced by other carbon related process, especially CO_2 interference form atmosphere, changes of Ca^{2+} are almost exclusively controlled by the $CaCO_3$ precipitation.

In summary, this method can be widely applied to the analysis of Ca^{2+} distribution in the ocean and the laboratory studies of calcium carbonate precipitation and dissolution in seawater. Determination of $[Ca^{2+}]$ in high precision can help to elucidate the precipitation/dissolution process of calcium carbonate, especially the process of being affected by biological

activity. High quality Ca^{2+} data can also be compared with DIC and TAlk dataset to describe the carbonate system in a more comprehensive way.

CONCLUSION

Based on the common method, a simple, high precision, non-toxic and labor-saving technique using IC for detecting Ca^{2+} concentration in seawater has been developed. With the manual sample injection method, an overall precision of better than 0.1% could be achieved in Ca^{2+} detection in seawater. Instead of the single calcium standard solution, IAPSO seawater is used as the Ca^{2+} reference to achieve a higher accuracy.

Effects of sample dilutions, Mg^{2+} and Sr^{2+} on measured $[Ca^{2+}]$ have also been investigated. Sample dilutions have no discernible effect on the measurements. However, due to the little overlap between peaks, Mg^{2+} shows a linear decrease in measured $[Ca^{2+}]$ with the increasing Mg/Ca ratio in seawater. Thus, according to the measured Mg/Ca ratio in seawater, the measured $[Ca^{2+}]$ can be further corrected to reach higher accuracy with deviation within 0.1%. Sr^{2+} has similar effects on the Ca^{2+} measurements and the results can also be corrected with a similar correction method.

This method has been applied to the analysis of Ca^{2+} distribution in Southwest Indian Ocean and the laboratory study on the calcite precipitation in seawater. The small variations of $[Ca^{2+}]$ can be effectively detected in the experiment of calcite precipitation in seawater and that are consistent well with the variations of DIC and TAlk. This shows that our method offers good sensitivity for Ca^{2+} determinations in seawater with high precision. It will contribute to a more comprehensive study of the carbonate system in seawater with Ca^{2+} parameter.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

AUTHOR CONTRIBUTIONS

YP directed the study and its scope. HH, YL, SW, and YP conceived and designed the experiments. HH, YL, SW, and QM performed the experiments. HH and SW compiled and processed all the field and laboratory data. HH wrote the manuscript, with edits and contributions from all co-authors.

FUNDING

ACKNOWLEDGMENTS

REFERENCES

Andersson, A. J., and Gledhill, D. (2013). Ocean acidification and coral reefs: effects on breakdown, dissolution, and net ecosystem calcification. *Ann. Rev. Mar. Sci.* 5, 321–348. doi: 10.1146/annurev-marine-121211-172241

Barrett, P. M., Resing, J. A., Buck, N. J., Feely, R. A., Bullister, J. L., et al. (2014). Calcium carbonate dissolution in the upper 1000 m of the eastern North Atlantic. *Glob. Biogeochem. Cycles* 28, 386–397. doi: 10.1002/2013gb004619

Cai, W. J., Hu, X., Huang, W. J., Murrell, M. C., Lehrter, J. C., et al. (2011). Acidification of subsurface coastal waters enhanced by eutrophication. *Nat. Geosci.* 4, 766–770. doi: 10.1038/ngeo1297

Caland, L. B. D., Silveira, E. L. C., and Tubino, M. (2012). Determination of sodium, potassium, calcium and magnesium cations in biodiesel by ion chromatography. *Anal. Chim. Acta* 718, 116–120. doi: 10.1016/j.aca.2011.12.062

Cao, Z., and Dai, M. (2011). Shallow-depth $CaCO_3$ dissolution: evidence from excess calcium in the South China Sea and its export to the Pacific Ocean. *Glob. Biogeochem. Cycles* 25:GB2019.

Chen, C. A. (1978). Decomposition of calcium carbonate and organic carbon in the deep oceans. *Science* 201, 735–736. doi: 10.1126/science.201.4357.735

Chung, S. N., Lee, K., Feely, R. A., Sabine, C. L., Millero, F. J., et al. (2003). Calcium carbonate budget in the Atlantic Ocean based on water column inorganic carbon chemistry. *Glob. Biogeochem. Cycles* 17:1093.

Culkin, F., and Cox, R. A. (1966). Sodium, potassium, magnesium, calcium and strontium in sea water. *Deep Sea Res. Oceanogr. Abst.* 13, 789–804. doi: 10.1016/0011-7471(76)90905-0

Dickson, A. G., Sabine, C. L., and Christian, J. R. (2007). *Guide to Best Practices for Ocean CO_2 Measurements*. Sidney: North Pacific Marine Science Organization.

Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A. (2009). Ocean acidification: the other CO_2 problem. *Annu. Rev. Mar. Sci.* 1, 169–192.

Feely, R. A., and Chen, C. T. A. (1982). The effect of excess CO_2 on the calculated calcite and aragonite saturation horizons in the northeast Pacific. *Geophys. Res. Lett.* 9, 1294–1297. doi: 10.1029/gl009i011p01294

Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., et al. (2004). Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science* 305, 362–366. doi: 10.1126/science.1097329

García-Fernández, R., Garcıá-Alonso, J. I., and Sanz-Medel, A. (2004). Simultaneous determination of inorganic anions, calcium and magnesium by suppressed ion chromatography. *J. Chromatogr. A* 1033, 127–133. doi: 10.1016/j.chroma.2004.01.024

Grasshoff, K., Kremling, K., and Ehrhardt, M. (2009). *Methods of Seawater Analysis*. Hoboken, NJ: John Wiley & Sons.

Hadjiioannou, T. P., and Papastathopoulous, D. S. (1970). EDTA titration of calcium and magnesium with a calcium-selective electrode. *Talanta* 17, 399–406. doi: 10.1016/0039-9140(70)80085-6

Horibe, Y., Endo, K., and Tsubota, H. (1974). Calcium in the South Pacific, and its correlation with carbonate alkalinity. *Earth Planet. Sci. Lett.* 23, 136–140. doi: 10.1016/0012-821x(74)90040-5

Jiang, L. Q., Cai, W. J., Feely, R. A., Wang, Y. C., Guo, X. H., et al. (2010). Carbonate mineral saturation states along the U.S. East Coast. *Limnol. Oceanogr.* 55, 2424–2432. doi: 10.4319/lo.2010.55.6.2424

Kanamori, S., and Ikegami, H. (1980). Computer-processed potentiometric titration for the determination of calcium and magnesium in sea water. *J. Oceanogr. Soc. Jpn.* 36, 177–184. doi: 10.1007/bf02070330

Kester, D. R., Duedall, I. W., Connors, D. N., and Pytkowicz, R. M. (1967). Preparation of artificial seawater. *Limnol. Oceanogr.* 12, 176–179.

Kleypas, J. A., Buddemeier, R. W., and Gattuso, J. P. (2001). The future of coral reefs in an age of global change. *Int. J. Earth Sci.* 90, 426–437. doi: 10.1007/s005310000125

Krumgalz, B. S. (1982). Calcium distribution in the world ocean waters. *Oceanol. Acta* 5, 121–128.

Laikhtman, M., Riviello, J., and Rohrer, J. S. (1998). Determination of magnesium and calcium in 30% sodium chloride by ion chromatography with on-line matrix elimination. *J. Chromatogr. A* 816, 282–285. doi: 10.1016/s0021-9673(98)00530-5

Lebel, J., and Poisson, A. (1976). Potentiometric determination of calcium and magnesium in seawater. *Mar. Chem.* 4, 321–332. doi: 10.1016/0304-4203(76)90018-9

Melendez, M., Corredor, J. E., Nesterenko, E. P., and Nesterenko, P. N. (2013). Direct chromatographic separation and determination of calcium and magnesium in seawater and sediment porewaters. *Limnol. Oceanogr. Methods* 11, 466–474. doi: 10.4319/lom.2013.11.466

Millero, F. J. (2013). *Chemical Oceanography, Fourth Edition*. Boca Raton, FL: CRC Press.

Millero, F. J., Feistel, R., Wright, D. G., and McDougall, T. J. (2008). The composition of standard Seawater and the definition of the reference-composition salinity scale. *Deep Sea Res. I* 55, 50–72. doi: 10.1016/j.dsr.2007.10.001

Millero, F. J., Zhang, J. Z., Lee, K., and Campbell, D. M. (1993). Titration alkalinity of seawater. *Mar. Chem.* 44, 153–165. doi: 10.1016/0304-4203(93)90200-8

Milliman, J. D., Troy, P. J., Balch, W. M., Adams, A. K., Li, Y. H., and Mackenzie, F. T. (1999). Biologically mediated dissolution of calcium carbonate above the chemical lysocline? *Deep Sea Res. I Oceanogr. Res. Pap.* 46, 1653–1669. doi: 10.1016/s0967-0637(99)00034-5

Naik, S., Noronha, R. J., Shirodkar, P. V., and Gupta, R. S. (1983). On the distribution of calcium, magnesium, sulphate and boron in the south-western Indian Ocean region of the Southern Ocean. *Sci. Rep. First Indian Exp. Antarct.* 1983, 87–94.

Olson, E. J., and Chen, C. A. (1982). Interference in the determination of calcium in seawater. *Limnol. Oceanogr.* 27, 375–380. doi: 10.4319/lo.1982.27.2.0375

Riley, J. P., and Tongudai, M. (1967). The major cation/chlorinity ratios in sea water. *Chem. Geol.* 2, 263–269. doi: 10.1016/0009-2541(67)90026-5

Sabine, C. L., Key, R. M., Feely, R. A., and Greeley, D. (2002). Inorganic carbon in the Indian Ocean: distribution and dissolution processes. *Glob. Biogeochem. Cycles* 16:1067.

Schmid, R. W., and Reilley, C. N. (1957). New complexon for titration of calcium in presence of magnesium. *Anal. Chem.* 29, 258–264.

Shiller, A. M., and Gieskes, J. M. (1980). Processes affecting the oceanic distributions of dissolved calcium and alkalinity. *J. Geophys. Res. Oceans* 85, 2719–2727.

Smith, S. V., and Mackenzie, F. T. (2016). The role of $CaCO_3$ reactions in the contemporary oceanic CO_2 cycle. *Aquat. Geochem.* 22, 153–175. doi: 10.1007/s10498-015-9282-y

Thomas, D. H., Rey, M., and Jackson, P. E. (2002). Determination of inorganic cations and ammonium in environmental waters by ion chromatography with a high-capacity cation-exchange column. *J. Chromatogr. A* 956, 181–186. doi: 10.1016/s0021-9673(02)00141-3

Tsunogai, S., Nishimura, M., and Nakaya, S. (1968). Calcium and magnesium in seawater and the ratio of calcium to chlorinity as a tracer of water masses. *J. Oceanogr. Soc. Jpn.* 24, 153–159. doi: 10.5928/kaiyou1942.24.153

Villiers, S. D. (1998). Excess dissolved Ca in the deep ocean: a hydrothermal hypothesis. *Earth Planet. Sci. Lett.* 164, 627–641. doi: 10.1016/s0012-821x(98)00232-5

Villiers, S. D. (1999). Seawater strontium and Sr/Ca variability in the Atlantic and Pacific oceans. *Earth Planet. Sci. Lett.* 171, 623–634. doi: 10.1016/s0012-821x(99)00174-0

Whitfield, M., and Leyendekkers, J. V. (1969). Liquid ion-exchange electrodes as end-point detectors in compleximetric titrations. Determination of calcium and magnesium in the presence of sodium. *Anal. Chim. Acta* 46, 63–70. doi: 10.1016/0003-2670(69)80042-5

Zeng, W., Chen, Y., Cui, H., Wu, F., Zhu, Y., and Fritz, J. S. (2006). Single-column method of ion chromatography for the determination of common cations and some transition metals. *J. Chromatogr. A* 1118, 68–72. doi: 10.1016/j.chroma.2006.01.065