

Direct chromatographic separation and quantification of calcium and magnesium in seawater and sediment porewaters

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Abstract

Direct analysis of Ca²⁺ and Mg²⁺ is required for accurate determination of metastable carbonate mineral phase saturation states (Ω_{CaCO_3} ; Ω_{MgCO_3}) in seawater, sediment porewaters, and other high ionic strength brines. To this end, we have implemented a method using High Performance Chelation Ion Chromatography (HPCIC) in which metal ion complexation at the stationary phase renders separation efficiency insensitive to high ionic strength matrix effects common to other ion chromatography (IC) methods. This method, using direct automated on-column injection, vastly increases sample throughput capacity in comparison to current titration methods. Calcium and magnesium ions in IAPSO standard seawater were selectively separated using a monolithic silica column (100 × 4.6 mm ID) activated with a covalently bonded iminodiacetic acid (IDA) chelator. The colored ion complexes resulting from post-column reaction (PCR) of the ions with a metallochromic indicator, in this case 4-(2-pyridylazo)-resorcinol (PAR), were detected spectrophotometrically at 510 nm. Optimization of flow rate, eluent concentration, pH, and sample injection volume allowed baseline separation of Mg²⁺ (0.05474 mol kg⁻¹) and Ca²⁺ (0.01065 mol kg⁻¹) in less than 8 min using 2 μ L seawater sample injections. At a flow rate of 1 mL min⁻¹, peak elutions occurred respectively at 4 and 5 min, using an eluent containing 0.1 M potassium chloride and 1 mM nitric acid adjusted to pH 2.5. Retention time variability below 0.5% for both metals following more than 200 injections indicates long-term stability of the derivatized monolithic silica column. Method application to marine sediment porewaters is discussed.

Accurate determination of Mg²⁺ and Ca²⁺ ion concentrations and their relative proportions in seawater, marine sediment porewaters and other environmental high ionic strength brines is troublesome despite their high concentrations due to the complexities of the matrix and chemical similarity which results in their co-precipitation (Traganza and Szabo 1967; Carpenter and Manella 1973; Kanamori and Ikegami 1980). Although Mg²⁺ and Ca²⁺ ion concentrations in open ocean waters are largely conservative with respect to salinity, coastal

processes including biogenic and abiogenic precipitation, as well as carbonate sediment dissolution, can result in deviations from this norm (Gledhill 2005; Ribou et al. 2007). During carbonate precipitation from seawater, Mg²⁺ for example, can co-precipitate with Ca²⁺ yielding high-magnesium calcite thereby altering the Ca²⁺/Mg²⁺ ratio and the ion activity product relative to Mg-calcite mineral phases. These Mg-calcite mineral phases (low and high Mg-calcites) are not well understood due to problems involving the precision of measurements and uncertainty regarding the basic thermodynamic solubility and kinetic properties of these phases (Morse et al. 2006). Accurate determination of Mg²⁺ and Ca²⁺ ion concentrations can help elucidate such difficulties.

Changes in seawater saturation state (Ω) with respect to metastable carbonate phases are not only affected by changes in the seawater CO₃²⁻ concentration. Variations in the Mg²⁺ and Ca²⁺ ion seawater concentrations and their ratio imply changes in Mg-calcite composition and solubility, Mg content in marine calcifying organisms skeletons, as well as changes in seawater Ω (Andersson et al. 2008). The delicate equilibrium between these two alkaline earth metal cations is triggered by

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slight changes in alkalinity and carbon dioxide tension that may cause their precipitation or dissolution (Laurence 1926; Brewer et al. 1975). In sediment porewaters, changes in Ca^{2+} , Mg^{2+} , and CO_3^{2-} concentrations arise from the precipitation or dissolution of calcium carbonate minerals (Koczy 1956; Traganza and Szabo 1967; Kanamori and Ikegami 1980; Kleypas et al. 2006; Ribou et al. 2007). The direct quantification of the Mg^{2+} and Ca^{2+} seawater ion concentrations can help in understanding the chemical behavior of seawater metastable carbonate mineral phases and in more accurate determination of their corresponding seawater Ω (Kleypas et al. 2006; Ribou et al. 2007).

Current methods for the determination of Mg^{2+} and Ca^{2+} ion concentrations in seawater use gravimetric procedures and ion-exchange separation combined with titration methods. Due to the precision difficulties, seawater Mg^{2+} ion concentration is usually determined as the difference between total alkaline earth metals and Ca^{2+} plus strontium (Kanamori and Ikegami 1980). Meanwhile Ca^{2+} is selectively titrated with Zincon (Zn-ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid [EGTA]) (Culkin and Cox 1976; Kanamori and Ikegami 1980), ethylenediamine-N,N,N',N'-tetraacetic acid (EDTA) (Riley and Tongudai 1967), or glyoxal-bis(2-hydroxyanil) (GBHA) (Tsunogai et al. 1968). Other methods incorporate ion selective electrodes as end-point indicators (Whitfield et al. 1969; Růžička et al. 1973; Lebel and Poisson 1976; Kanamori and Ikegami 1980). These methods are laborious and time-consuming, and as a result, sample throughput is limited in the best cases to a few tens of sample analyses per day. Additionally, most of these techniques do not have sufficient resolution to detect the small changes due to calcification processes. A direct in situ method using a custom-made ion-selective electrode has been described (Wenzhöfer et al. 2001), but resolution is poor and the electrode is not commercially available. Other instrumental methods include inductively coupled plasma spectrometry (ICP-MS), atomic absorption spectrophotometry (AAS), and flame atomic absorption spectrophotometry (FAA). However sample pretreatment is needed, interferences from other major ionic components in the matrix are expected, and analysis costs can be high.

Recently, chromatographic techniques have been developed that can provide higher energy interactions between the ionic analytes of interest and selected adsorbents or stationary phases increasing significantly the degree of separation selectivity. Chelation ion chromatography (CIC), first described by Moyers and Fritz in 1977, is a retention mechanism that allows specific interactions between a dissolved metal ion analyte and a chelating stationary phase. Paull et al. (1996) demonstrated the potential application of CIC to the problem of Mg^{2+} and Ca^{2+} ion separation using a dynamic chelating ion exchange mechanism whereby a chelator dissolved in the carrier coats a porous graphitic carbon column. In recent developments, selected organic ligands covalently bonded to inert

substrates serve as the stationary phase. Metal ion analytes form very stable complexes with these ligands and hence efficient separation is achieved (Nesterenko et al. 2011; Nesterenko et al. 2013). The use of chelating ion-exchangers to form kinetically labile surface complexes and retain metal ions according to the stability of corresponding complexes is one of the multiple advantages in high performance liquid chelation ion chromatography (HPLCIC) (Nesterenko and Jones 2007). Modification of monolithic silica columns with covalently bonded chelating iminodiacetic acid (IDA) groups has proven to allow excellent cation separation and increased peak efficiencies compared with other columns.

We here describe implementation of an HPLCIC method for separation and quantification of Mg^{2+} and Ca^{2+} ions in seawater in less than 8 min. We use a monolithic silica column derivatized with a covalently bonded IDA chelator for separation, and post-column derivatization of the ions with 4-(2-pyridylazo)-resorcinol (PAR) for optical detection and quantification. The metallochromic reagent PAR forms water-soluble complexes with Mg^{2+} and Ca^{2+} ions of moderate molar absorptivities ($\sim 10^4$ at about 500 nm), therefore exhibiting robust sensitivity for spectrophotometric detection (Jezorek and Freiser 1979). Monolithic HPLC columns, employing a continuous silica matrix etched with porous channels, surpass traditional packed bead column performance with higher separation efficiency, reduced retention times, and low column backpressure.

Benefits of this method include elimination of the need for sample pretreatment or manipulation, high sample throughput achievable with automated sample injection, low sample volume required, reduced number of solutions necessary, lack of interference from other ionic compounds, method simplicity and reliability, and reduced sensitivity to the ionic strength of the sample matrix. Whereas we have yet to achieve the canonical precision of 0.1% quoted for seawater applications (Carpenter and Manella 1973; Kanamori and Ikegami 1980; Olson and Chen 1982), the method can currently be applied to sediment porewaters and further method refinement is expected to achieve this requirement.

To explore anticipated improvement in method reproducibility with increased injection volume but given limitation to seawater Ca^{2+} and Mg^{2+} analysis imposed by column-loading capacity and detector saturation, we performed a series of experiments using increasing injection volumes of Mn^{2+} ion proxy at low concentration. Manganese ion was chosen because it exhibits greater molar absorptivity with the PAR reagent allowing use of more dilute and less acidic solutions.

Materials and procedures

Monolithic silica IDA modified column

A monolithic bare silica column (Phenomenex 100 \times 4.6 mm) was modified with IDA chelator through the activation of silanol groups at the surface of the silica monolith column with distilled water at 60°C followed by recycling of mixture

IDA and 3-glycidoxypolypropyltriethoxysilane through the column at 70°C (for method details see Sugrue et al. 2003; Nesterenko and Jones 2007; Nesterenko et al. 2013). Surface treatment and functionalization of the continuous unitary porous structure and structure of the bonded layer within such columns have been described by Sugrue et al. (2004) and Nesterenko et al. (2013).

Reagents and solutions

For photometric detection, we used PAR reagent (CAS# 1141-59-9, acid form – Fluka, 99% purity) as a post-column reagent. We prepared stock solutions of 1 mM PAR and 2 M ammonium hydroxide (analytical reagent grade). The high pH of the stock solution prevents adsorption onto plastic surfaces. The standard post column reagent was prepared by dilution to 0.05 mM PAR. To adjust the pH to ~ 10.4, we used 2 M nitric acid (analytical reagent grade). The post-column reagent thus prepared is stable for weeks if not months, and will not need filtering, degassing, or an overpressure of inert gas.

The mobile phase was prepared using 0.1 M potassium chloride (KCl) and 1 mM HNO₃, pH of ~ 2.5. Standard seawater (International Association for the Physical Sciences of the Ocean – IAPSO, batch 149; 10 May 2007) with salinity 34.994 was purchased from OSIL (Havant, UK). Stoichiometric reference composition of IAPSO standard seawater provides the best current estimation of Mg²⁺ (0.05474 mol kg⁻¹) and Ca²⁺ (0.01065 mol kg⁻¹) concentrations in seawater (Millero et al. 2008).

We used Nalgene bottles for storage of all stock and working solutions due to their low metal contamination. Glassware and plasticware were acid washed before use with 10 mL of 1 M nitric acid followed by a rinse with deionized water provided from a Milli-Q system (Millipore, Bedford, USA).

Chromatographic instrumentation

A Waters 2695 HPLC Separations Module (Waters, Milford, MA, USA) chromatography system was used. The autosampler built in to the Separation Module allowed runs of 178 samples in a single analytical sequence. Column oven was set to 30°C for all separations. A post column reaction (PCR) flow system was used to allow cation detection. The 1/16" polypropylene mixing coil used in the PCR was about 2.5 m long using a high-pressure pump (Model 350 Scientific System Inc.). The colored PAR-derivatized cations were detected spectrophotometrically using a model 2487 UV/VIS spectrophotometric detector operated at 510 nm. Data were processed using the Waters Empower 3 Software.

Porewater samples

Stainless steel well samplers (3/4-inch ID) were developed, which allow porewater sampling down to 20 cm sediment depth. Each sampler is placed 1 m apart on a 10 m transect along the reef. Each sampling port consists of thirty 1/16-inch holes drilled around the sample in a 1 cm span. Samples were taken at 2 cm resolution through the upper 20 cm of the sediment column. Following the technique described by Falter and Sansone (2000), we installed the samplers by first hammering a stainless steel tube (54 cm long, 5/8-inch ID) into the sedi-

ment and then replacing it with the well sampler. After insertion into the sediment, the sampler was left on site allowing repetitive sampling at identical locations and depth intervals. Porewater samples were collected in situ by withdrawing porewater using two 60 cc syringes and storing in 125 mL plastic sample bottles. Each sample was filtered through 0.45 µm membrane filters and poisoned with 60 µL of a saturated HgCl₂ solution to prevent biological alteration of the sample. Porewater salinity was determined using the Guildline Autosol 8400B salinometer with a precision of ± 0.003. Conductivity, Temperature, and Depth (CTD) casts of the overlying water column were routinely performed. Surface and bottom samples of the overlying seawater were collected for analyses as well using a Van Dorn bottle.

Assessments

Optimization of the method

We tested eluent concentration over ranges of 0.1 to 0.5 M KCl and 1 to 4 mM HNO₃ with pH between 2.5 and 3.0. Baseline cation separation and peak shape were found to be optimal at 0.1 M of KCl and 1 mM HNO₃. Systematic reduction of HNO₃ and KCl concentrations improved the response and produced sharper and narrower peak shapes. These optimized eluent concentrations allowed return to baseline between peaks for up to 0.5 s. Variations in eluent pH from 2 to 3 were tested, but no significant changes in retention or peak shapes were observed.

The effect on reaction completion of varying PCR reagent flow rate was tested. Increasing PCR reagent flow rate from 0.7 to 1 mL min⁻¹ resulted in increased photometric response for both analytes in standard seawater (Fig. 1). Maximum absorbance response was obtained at a flow rate of 1 mL min⁻¹. To minimize the ambiguities introduced in addressing both the reagent and eluent delivery proportions, absorbance

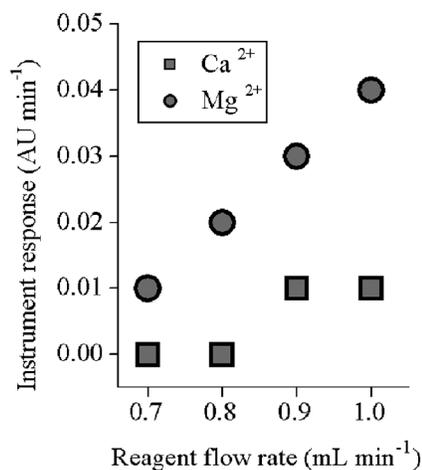


Fig. 1. Effect of PCR reagent flow rates on Ca²⁺ and Mg²⁺ responses using standard seawater as a probe. Maximum absorbance response is observed at 1 mL min⁻¹.

response was investigated through standard addition (Sugrue et al. 2003). Reagent flow rates tested ranged from 0.5 to 1 mL min⁻¹. Table 1 shows the changes in linear regression coefficients with variation of PCR flow rate of Mg²⁺ and Ca²⁺ ions. Peak absorbance exhibits a linear relationship with Ca²⁺ ion concentration ($R^2 = 0.99$, $n = 8$) at reagent flow rate of 0.5 mL min⁻¹, but Mg²⁺ is incompletely derivatized at this low reagent flow rate. The correlation coefficient for magnesium increased significantly with increased reagent flow rate. Highest linear correlation between reagent flow rate and absorbance response for Mg²⁺ was achieved at 0.9 mL min⁻¹ ($R^2 = 0.98$, $n = 10$). Despite a modest concurrent Ca²⁺ coefficient decrease

Table 1. Coefficients of determination of linear regressions (R^2) of Mg²⁺ and Ca²⁺ standard concentrations versus absorbance at different reagent flow rates using standard additions.

Reagent flow rate (mL min ⁻¹)	R^2 Mg ²⁺	R^2 Ca ²⁺
0.5	0.05	0.99
0.6	0.23	0.97
0.7	0.51	0.96
0.8	0.73	0.82
0.9	0.98	0.95

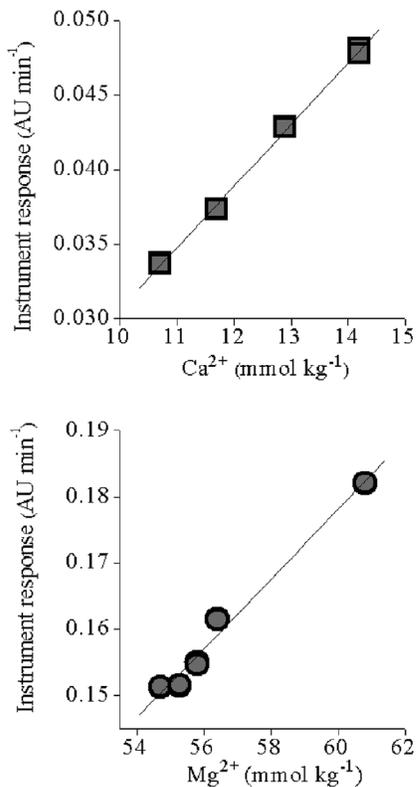


Fig. 2. Linearity of Ca²⁺ ($R^2 = 0.99$, $n = 8$) and Mg²⁺ ($R^2 = 0.98$, $n = 10$) standard addition at reagent flow rate of 0.9 mL min⁻¹. Standards were measured in duplicate.

from 0.99 to 0.95, this does not significantly compromise analyte determination. Fig. 2 shows the dependence of response against concentration of both metals using different standard addition concentrations of Mg²⁺ and Ca²⁺, at 1 mL min⁻¹ eluent flow rate and 0.9 mL min⁻¹ PCR reagent flow rate.

Effect of sample injection volume was tested for 2, 5, and 10 μ L by assessing replicate reproducibility of three standard seawater injections ($n = 9$). Increasing sample volume resulted in lower reproducibility as shown by the increased standard deviation of peak areas (Fig. 3). Response reproducibility was best with smallest injected volume of the sample. Injection volume of 2 μ L was consequently selected for routine operation.

Increased detector response with increased PRC reagent flow rate, but the absence of plateaus for either Ca²⁺ or Mg²⁺ (Fig. 1), indicates that post-column reaction completion was not reached even at the highest flow rate possible (1 mL min⁻¹). These circumstances result in limited method reproducibility despite intentional minimization of injection volume to the smallest injection loop possible (1 mL).

To explore the effect of varying sample injection volume on reproducibility using the Mn²⁺ ion proxy (given the column loading limitation for Ca²⁺ and Mg²⁺ ions) at a concentration of 45.5 nM prepared from standard 1000 ppm in 0.5 M nitric acid, we performed 10 consecutive runs each for 2, 4, 10, and 20 μ L injection volumes keeping column temperature and sample temperature constant at 25°C. The eluent used in this test was slightly different (3 mM HNO₃; 0.1 M KCl) from that used for seawater Mg²⁺ and Ca²⁺ analysis to minimize run time. The post column reagent was as previously described delivered at 0.9 mL min⁻¹. Increased injection volume using the Mn²⁺ ion proxy resulted in reduction of the relative standard deviation (RSD) from 4.8% at 2 μ L injection volume to 0.7% at 20 μ L injection volume (Fig. 4).

Method performance following optimization

A chromatogram performed under optimized parameters using the IDA-modified silica monolithic column shows com-

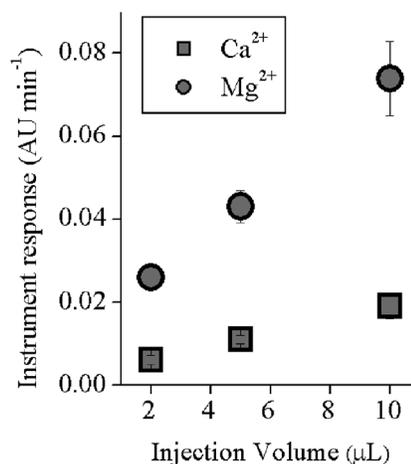


Fig. 3. Effect of sample volume injection on relative instrument response.

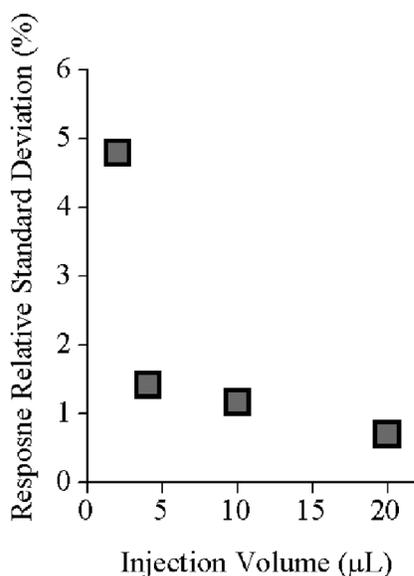


Fig. 4. Effect of increasing sample injection volumes on method reproducibility using Mn^{2+} metal as an ion proxy.

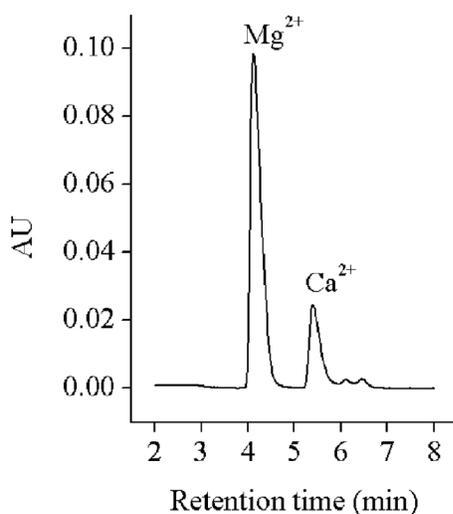


Fig. 5. Chromatogram of Mg^{2+} and Ca^{2+} in standard seawater obtained using eluent of 0.1 M of KCl and 1 mM of HNO_3 at pH ~ 2.5, flow-rate, 1.0 mL min^{-1} ; sample injection volume, 2 μL ; PCR 0.05 mM PAR at pH ~ 10.4, flow rate, 0.9 mL min^{-1} ; photometric detection at 510 nm.

plete baseline separation of Mg^{2+} and Ca^{2+} ions in standard seawater achieved in less than 8 min at a flow rate of 0.9 mL min^{-1} (Fig. 5). Column efficiencies calculated from chromatographic peaks are 13,720 and 24,800 theoretical plates per meter for Mg^{2+} and Ca^{2+} , correspondingly. These numbers are in a good agreement with values ranging 18,000 to 37,560 reported for such columns (Sugrue et al. 2003). The difference in efficiency calculated for the massive Mg^{2+} peak is connected with partial overloading of the column, which caused some peak broadening. Although increased eluent flow rate can

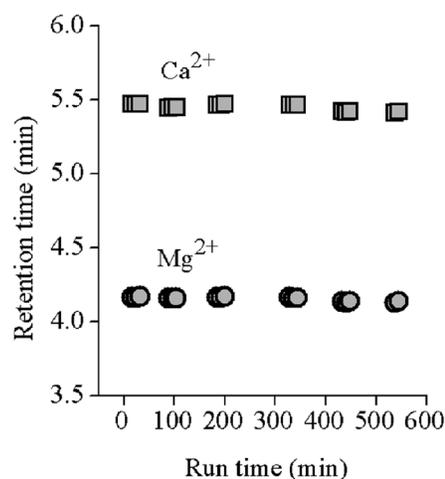


Fig. 6. Column stability as indicated by retention time consistency for Mg^{2+} and Ca^{2+} in standard triplicate seawater samples analyzed following every ~ 30 porewater sample injections.

deliver separation in a period of less than 4 min, selectivity, peak symmetry, and resolution of the massive Ca^{2+} and Mg^{2+} peaks in our samples are favored at the lower flow rate with the higher retention time.

Retention time variability was used to assess long-term column stability. During operational runs, a sample of standard seawater was analyzed every ~ 30 injections. Twenty-one samples of standard seawater were analyzed through the sequence of 60 sediment porewater samples. The average retention times were $4.15 \pm 0.01 \text{ min}$ for Mg^{2+} and $5.44 \pm 0.02 \text{ min}$ for Ca^{2+} . Retention time variability over the 656 min (10.9 h) of consecutive injections was 0.4% and 0.5%, respectively, for Mg^{2+} and Ca^{2+} (Fig. 6).

Throughout a sequence of 243 consecutive sample injections, the maximum variability from the stoichiometric reference composition of standard seawater defined by Millero et al. (2008) (Mg^{2+} $0.05474 \text{ mol kg}^{-1}$ and Ca^{2+} $0.01065 \text{ mol kg}^{-1}$) was 1% for Mg^{2+} and 2% for Ca^{2+} (Fig. 7 and Table 2).

Determination of Mg and Ca in sediment porewaters

Example of method application

The optimized HPCIC method enabled analysis of high ionic strength seawater and porewaters samples. Vertical distribution and temporal variability of Mg^{2+} and Ca^{2+} in sediment porewaters collected at a mid-shelf reef off La Parguera, Puerto Rico, from June to September 2011 was examined. We analyzed a series of 60 samples in triplicate (180 injections) under optimized conditions as described above. Average RSD for triplicate samples was 1% and 2%, respectively, for Mg^{2+} and Ca^{2+} ions. The maximum and the minimum RSD registered for one sample in triplicate was 2% and 7% and 0.1% and 0.2%, respectively, for Mg^{2+} and Ca^{2+} ions.

In general, Mg^{2+} and Ca^{2+} sediment porewater concentrations at Enrique Reef increased with depth in the sediment column. Whereas temporal changes are apparent, no definite tem-

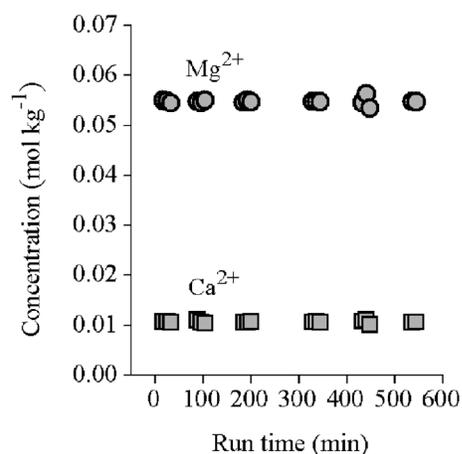


Fig. 7. Column stability as indicated by the concentration of Mg^{2+} and Ca^{2+} in standard seawater analyzed following every ~ 30 porewater sample injections.

poral trend was evident (Fig. 8). The maximum increases over sediment-water interface surface values to 16 cm depth within the sediment were 0.0063 and 0.0038 mol kg^{-1} for Mg^{2+} and Ca^{2+} , respectively. These changes with depth are large relative to the measurement error. Porewater Mg^{2+}/Ca^{2+} ratios decreased with depth presumably as a result of sediment dissolution of metastable carbonate phases. Maximum and minimum Mg^{2+}/Ca^{2+} ratios were 5.37 and 4.22, respectively (Fig. 9).

Discussion

The method here described optimizes chelation-based separation of the alkaline earth metal ions Mg^{2+} and Ca^{2+} at high concentrations on the monolithic IDA column using a high ionic strength/low pH eluent. The method makes possible rapid automated analysis of Mg^{2+} and Ca^{2+} in high ionic strength matrices such as marine sediment porewaters.

Completion of the post-column complexation reaction with the colored reagent posed an analytical challenge due to the high concentration of Mg^{2+} and Ca^{2+} , third and fourth most abundant ions in seawater. Magnesium was a particular challenge because of its high concentration in seawater and mainly because of its short residence time in the chromatographic column. The latter factor means that the Mg^{2+} band migrates through a significant part of the chromatographic column together with the massive band of alkali metal cations from seawater resulting in column overloading and peak broadening. Kinetically, complex formation with PAR was “fast.” Increasing the PCR reagent delivery allowed the complexes to form through the post-column reaction. This process is apparent in the resulting chromatogram (Fig. 5), which because of the fast kinetics of chelate formation and dissociation shows relatively narrow peak shapes.

Column efficiency was not compromised throughout the analytical run of 243 samples, and no significant variability of retention times was observed (see Fig. 6). The use of covalently

Table 2. Variability of apparent Mg^{2+} and Ca^{2+} ion concentrations and ionic ratio in standard seawater (SSW) analyzed throughout the porewater sample analysis run. Percent difference from Mg^{2+} and Ca^{2+} ion concentrations in standard seawater as established by Millero et al. (2008).

<i>n</i>	[Mg^{2+}] (mol kg^{-1})	[Ca^{2+}] (mol kg^{-1})	% Delta Mg^{2+} to SSW	% Delta Ca^{2+} to SSW
1	0.0550	0.0107	0	0
2	0.0548	0.0107	0	0
3	0.0544	0.0106	-1	-1
4	0.0547	0.0111	0	4
5	0.0545	0.0105	0	-1
6	0.0550	0.0104	0	-3
7	0.0546	0.0106	0	-1
8	0.0550	0.0106	0	0
9	0.0547	0.0108	0	1
10	0.0548	0.0107	0	0
11	0.0547	0.0107	0	0
12	0.0547	0.0106	0	-1
13	0.0545	0.0108	0	1
14	0.0563	0.0110	3	3
15	0.0534	0.0102	-2	-4
16	0.0548	0.0106	0	0
17	0.0547	0.0107	0	0
18	0.0549	0.0106	0	0
19	0.0546	0.0106	0	0
20	0.0547	0.0107	0	1
Avg	0.0547	0.0107	0	0
SD	0.0005	0.0002	1	2

bonded chelating reagents in the stationary phase of the monolithic column reduced the necessity for dilution, sample pre-treatment, or the use of multi-column separation techniques. The high retention of Mg^{2+} and Ca^{2+} on the surface monolithic phase is evident. The column can be used to analyze other alkaline earth metals, such as Sr^{2+} and Ba^{2+} in samples containing excess Mg^{2+} and Ca^{2+} (Sugrue et al. 2003; Nesterenko et al. 2013).

The method using the IDA-modified silica monolithic column described in this study offers new possibilities to gain meaningful insight into the biogeochemical processes occurring in permeable sediments. Organic matter remineralization processes and the concomitant metabolic CO_2 production force carbonate dissolution in aerobic surface layers of the calcareous marine sediments resulting in increased Mg^{2+} and Ca^{2+} concentrations at depth within the sediment (Burdige and Zimmerman 2002; Andersson et al. 2006). Direct porewater Mg^{2+} and Ca^{2+} ion measurements provide additional evidence for the occurrence of sediment carbonate dissolution and can be used to address the question of preferential dissolution of metastable carbonate phases (Mackenzie et al. 1983; Morse et al. 1985, 2006; Burdige and Zimmerman 2002).

System limitations for handling the massive alkaline earth cation concentrations of seawater remain. Addressing the

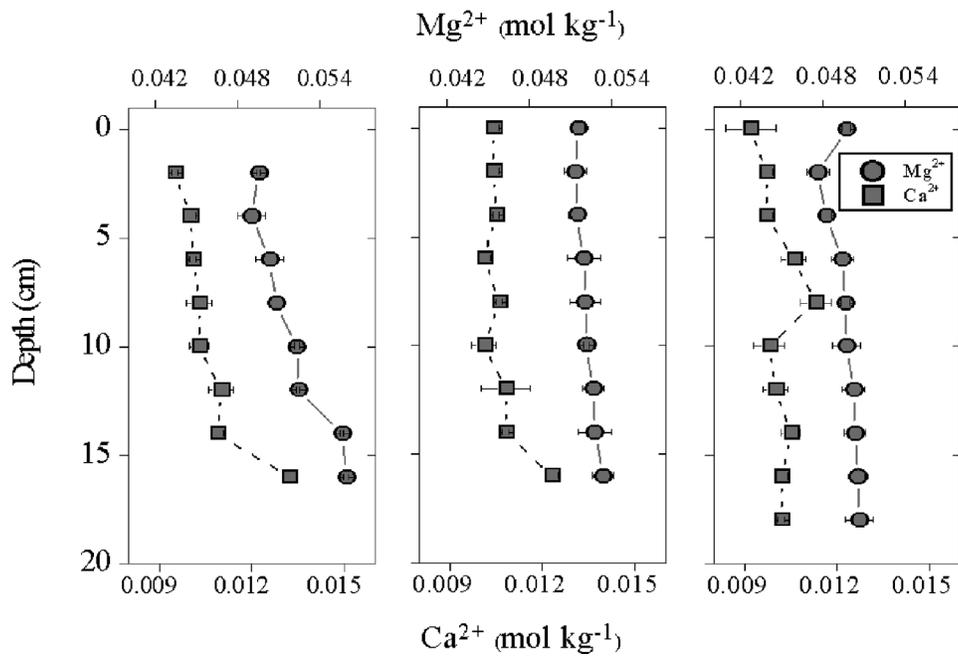


Fig. 8. Vertical porewater profiles for Mg²⁺ and Ca²⁺ ion concentration at Enrique Reef during June (left), July (center), and September 2011 (right).

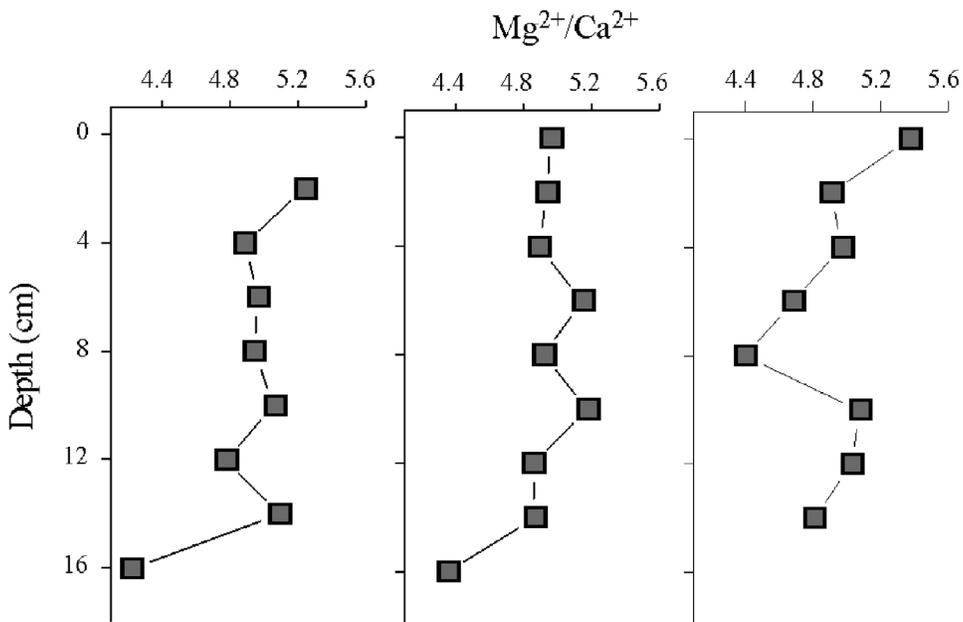


Fig. 9. Vertical porewater profiles for Mg²⁺/Ca²⁺ ion ratios at Enrique Reef during June (left), July (center), and September 2011 (right).

“chemical” problems of column overload, detector saturation, and reaction completion by sample volume reduction resulted in the “mechanical” problem of poor injection reproducibility. To confirm the dependence of reproducibility on injection sample volume, we used a very dilute Mn²⁺ solution so as to assure operation within the linear range of the calibration plot. Dramatic improvement of reproducibility with larger

injected volumes (up to 20 µL) (see Fig. 4) confirms the poor performance of low volume sample injection and points the way toward method optimization.

Comments and recommendations

Although the method as here presented is applicable to study large variations of Mg²⁺ and Ca²⁺ in marine sediment

porewaters, further improvement of method precision will be necessary for the determination of small changes in seawater. Observations of alkalinity changes indicate that calcification in coral reef environments can be expected to change Ca^{2+} concentration by less than ~ 0.050 mM over a daily cycle posing a considerable analytical challenge.

Accuracy of our analyses was compromised by the inability to reduce sample injection below $1 \mu\text{L}$ since we injected very concentrated samples into a column of internal diameter 4 mm. Our experiments with the Mn^{2+} ion proxy, however, demonstrate significant improvement of method reproducibility using greater injection volume (Fig.4). We consequently recommend the use of higher capacity columns (internal diameter 8 mm for example) to allow increasing sample volume injection to more reproducible volumes in the range of 10-20 μL . Auto sampler injection accuracy, in particular, increases substantially in this range.

Fully automated chromatographic analysis of Mg^{2+} and Ca^{2+} in seawater exhibits significant advantages over titration methods including rapid sample throughput, low sample volume, and decreased operator labor. A single chromatographic determination is 5 to 10 times faster than the corresponding determination by titration. Although autotitrators can be equipped with autosamplers, sample volumes used are orders of magnitude higher than for HPCIC resulting in lower sample loading capacity. Because the cost of the basic equipment, reagents, and materials is comparable for both methods, chromatographic determination is more cost-effective, especially for large sample numbers.

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