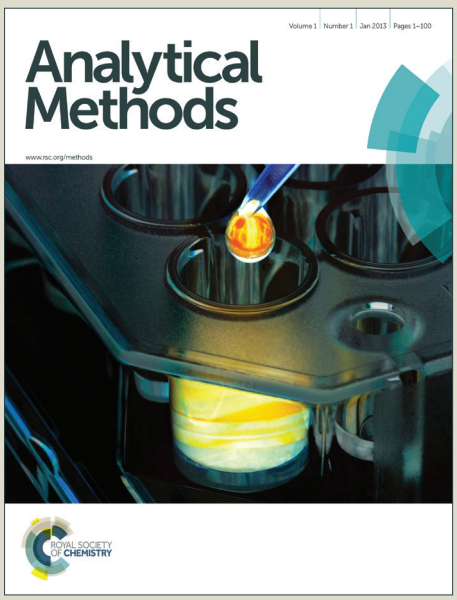
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Analytical Methods

RSCPublishing

ARTICLE

Determination of trace magnesium and strontium in calcium carbonate and calcareous skeletons of marine planktonic organisms using high performance chelation ion chromatography

Cite this: DOI: 10.1039/xoxxoooooox

Received ooth January 2014,
Accepted ooth January 2014

DOI: 10.1039/xoxxoooooox

www.rsc.org/

Yan Li[a], Brett Paull[a], Marius N. Müller[b,c] and Pavel N. Nesterenko[a,*]

A new high performance chelation ion chromatography method for the simultaneous determination of trace magnesium and strontium in various calcium carbonate samples was developed. Separations were performed on a monolithic silica column (Chromolith Si, 100 x 4.6 mm I.D.) chemically modified with hydroxyethyliminodiacetic acid functional groups. At a flow rate of 1.0 mL min^{-1}, an eluent containing 80 m*M* NaCl, 20 m*M* picolinic acid, pH 5.30, was found to provide complete separation of Ni^{2+}, Cu^{2+}, Mg^{2+}, Cd^{2+}, Sr^{2+} and Ca^{2+} in 15 minutes, in matrices with 20,000 fold excess of Ca^{2+}. Two post-column reagents, *o*-cresolphthalein complexone (*o*-CPC) and ZnEDTA-PAR, were compared for post-column reaction based photometric detection at 570 and 490 nm, respectively. This method provides sensitive detection of Mg^{2+} (LOD 20 µg L^{-1} and 5 µg L^{-1} for o-CPC and ZnEDTA-PAR, respectively) and Sr^{2+} (LOD 200 µg L^{-1} and 39 µg L^{-1} for o-CPC and ZnEDTA-PAR, respectively). Using *o*-CPC, linear range was from 0.5 to 24 mg L^{-1} for Mg^{2+} and from 1.0 to 32 mg L^{-1} for Sr^{2+}. For ZnEDTA-PAR, the linear range was from 0.5 to 4 mg L^{-1} for Mg^{2+} and 0.1 to 32 mg L^{-1} for Sr^{2+}. The method was applied to the analysis of a variety of calcium carbonate samples, including laboratory reagents, limestone NIST certified reference material, and the calcite based shells of marine microorganisms. Accuracy of the method was confirmed using inductively coupled plasma – mass spectrometry.

Introduction

Determination of trace metal ions in calcium carbonate matrices (commonly calcite) is of significant interest within a variety of scientific fields, particularly within geological and environmental studies. For example, limestone and dolomite diagenesis is known to be related to salinity and the Mg/Ca ratio[1]. Additionally, the trace metal chemistry of calcareous skeletons of marine microorganisms, such as coccolithophore calcite[2,3], can be utilised to provide insight into the nature of ancient oceans and environments. Furthermore, the rate of inorganic minor element incorporation (*e.g.* Mg and Sr), in calcium enriched matrices such as coccolithophore calcite, can be of great significance for the evaluation of the changes in current marine environment[4], including changes associated with global warming and increasing CO_2 emissions. However, in the latter case, complicating factors associated with removal of residue sea salts and organically bound or organic magnesium from coccolithophore calcite samples[3], means that currently there are few practical analytical methods for the accurate determination of trace inorganic magnesium, present as $MgCO_3$, in such materials. In addition, the excess of C and Ca within all forms of calcite can cause significant isobaric interferences for the precise determination of trace Mg^{2+} and Sr^{2+} (*e.g.* limestone[5]) when using techniques such as inductively coupled plasma mass spectrometry (ICP-MS)[6]. The measurement of magnesium isotopes ($^{24}Mg^+$, $^{25}Mg^+$ and $^{26}Mg^+$) can suffer isobaric interference from carbon-dimer ions ($^{12}C_2^+$, $^{12}C^{13}C^+$ and $^{13}C_2^+$). Similarly, strontium isotopes, ^{84}Sr, ^{86}Sr, ^{87}Sr and ^{88}Sr, can suffer isobaric interference from Ca-dimer ions signals ($^{40}Ca^{44}Ca^+$, $^{42}Ca_2^+$, $^{40}Ca^{46}Ca^+$, $^{42}Ca^{44}Ca^+$, $^{43}Ca_2^+$, $^{40}Ca^{48}Ca^+$, $^{42}Ca^{46}Ca^+$, and $^{44}Ca_2^+$). To fully eliminate such interferences, it is often necessary to apply additional chromatographic separations of these elements prior to introduction into the ICP-MS. For example, Chang *et al*. determined magnesium in $CaCO_3$[7] using multiple collector ICP-MS, by including a two-step ion-exchange chromatographic pre-treatment procedure. Likewise, extraction chromatography with polyacrylate porous resin coated by solution of 4,4'(5')-di-tert-butylcyclohexano-18-crown-6 in

octanol-1 was applied in a similar study for the determination of strontium[8,9]. Obviously, such coupled methods are rather complicated, expensive, and time consuming. Furthermore, the low recovery of elements following the separation procedure can also present a potential problem.

Other methods have also been reported for the determination of traces of magnesium or strontium in calcite, such as flame photometry[10], neutron activation analysis (NAA) [11] and atomic absorption spectroscopy (AAS)[12,13]. However, these methods also have their limitations. For example, the linear range of both atomic emission and absorption spectroscopy is rather narrow[13,14], and due to the high ionic strength and complexity of sample, analysis generally requires the removal of matrix and/or dilution.

Thus, alternative accurate and practical methods for the determination of Mg and Sr in calcium carbonate based matrices are in significant demand. Ion chromatography (IC) has obvious potential for such samples[15], and has been widely used for the determination of Mg and Sr in various complex sample matrices, including seawater[16], plant-tissues[17] and ceramic superconductors[18]. However standard IC, using traditional cation-exchange columns, suffers considerably when applied to samples of high ionic strength or those containing very diverse ion ratios, such as magnesium in concentrated calcium carbonate. To overcome these issues, high performance chelation ion chromatography (HPCIC) was developed, and specifically presented as a solution to the determination of alkaline earth and transition metal ions in such complex samples. The separation mechanism of HPCIC includes the formation of kinetically labile complexes between chelating groups and the sample metal ions, so that their retention and separation is not affected by the ionic strength of the samples[19,20]. Generally, chelating ligands are conjugate bases, resulting in their strong affinity to hydrogen ions. As a result of this affinity, pH can be used for control the separation selectivity and time.

The determination of alkaline earth metals in complex samples is one of the most effective applications of HPCIC. As far back as 1994, Jones etc.,[21] published the determination of Ba^{2+} and Sr^{2+} in calcium-containing matrices using HPCIC. However, this chromatographic system could not separate Mg^{2+} from the huge amount of Ca^{2+}. In addition, due to the infancy of the technique at the time (and column limitations), the whole separation took an excessive 50 minutes. Recent works reported much faster (5-15 min) determination of Be^{2+} in stream sediments[22], Mg^{2+}, Ca^{2+} and Sr^{2+} in seawater[6,23,24] and in saturated brines for the chlor-alkali industry[19].

The current work presents the results of determination of trace magnesium (and strontium) in calcium carbonate based matrices, such as certified argillaceous limestone, using HPCIC. The HPCIC methodology developed is based upon hydroxyethyliminodiacetic acid (HEIDA) bonded monolithic silica columns and optimised post-column reaction (PCR) detection. Applicability of the method to the analysis of calcareous skeletons of marine microorganisms, such as coccolithophores (in particular *Emiliania huxleyi*) is also demonstrated, with its potential as a new method for organic magnesium determination in coccolithic calcite discussed.

Experimental

Instrumentation

A Metrohm Model 844 Compact IC was used throughout this study. This system comprised of a built-in high pressure pump, peristaltic pump, UV-Vis detector and post-column reactor (Metrohm, Herisau, Switzerland). The post-column reactor consisted of a plastic tee and 2.5 m long PTEE capillary reaction coil. Two polyetheretherketone (PEEK) sample loops were used, 10 µL and 20 µL, respectively, with manual sample injection. ICNet 2.3 SR6 software (Metrohm, Herisau, Switzerland) was used for IC data acquisition and processing of chromatograms. A 100 × 4.6 mm I.D. Onyx silica monolithic column was purchased from Phenomenex (Cheshire, UK) and modified with HEIDA functional groups according to the procedure described elsewhere[25]. High-resolution (sector field) inductively coupled plasma mass spectrometry (HR-ICP-MS) was applied as a confirmatory method, using an ELEMENT 2 instrument (Thermo Fisher, Bremen, Germany).

Chemicals and reagent

Analytical or higher grade reagents and Milli-Q water (Millipore, Bedford, MA, USA) were used for preparing all solutions. *o*-Cresolphthalein complexone (*o*-CPC, 90% dye content) was sourced from Fluka (Buchs, Switzerland). 4-(2-pyridylazo) resorcinol (PAR) (99.5% dye content) was obtained from Sigma-Aldrich (Sydney, Australia). Picolinic acid (pyridine-2-carboxylic acid, 99%), dipicolinic acid (pyridine-2, 6-dicarboxylic acid, 99%) and sodium chloride (NaCl, 99.5%) were purchased from Sigma-Aldrich (Sydney, Australia). Sodium tetraborate ($Na_2B_4O_7{\cdot}10H_2O$, 99.5%), and Zn-EDTA were purchased from BDH chemicals (Poole, UK). $CaCO_3$ was purchased from BDH chemicals (Poole, UK), Strem Chemicals (Miami, USA) and AJAX chemicals (Sydney, Australia). Nitric acid (69%) and ammonium hydroxide (25%) were obtained from Merck (Sydney, Australia). Boric acid and Spectrosol atomic absorption standard solutions of Ni^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Sr^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+}, Zn^{2+} La^{3+} and Cu^{2+}, with concentrations of 1.00 g L^{-1} were purchased from BDH Chemicals (Poole, UK).

A certified reference sample (1d Limestone (Argillaceous)) was purchased from (National Institute of Standards and Technology, NIST, USA). Full details of its composition can be found at web-page http:/www.nist.gov/srm.

Preparation of post-column reagents

Three PCR reagents for the detection of alkaline earth metal cations and transition metal cations were used: (1) 0.4 m*M* *o*-CPC, 0.25 *M* boric acid adjusted to pH 11 using NaOH; (2) 0.15 m*M* PAR, 0.4 *M* NH_4OH, adjusted to pH 10.65 with nitric acid; (3) 0.2 m*M* ZnEDTA and 0.15 m*M* PAR in 2 *M* NH_4OH, adjusted to pH 10.65.

Sample preparation

For $CaCO_3$ and limestone samples, nitric acid (30 m*M*, 5 - 50 mL) was used to dissolve sample solid (50 mg) for 40 minutes under ultra-sonication. The final pH of the samples was adjusted to 2.0 – 3.0 to ensure all the carbonates were fully dissolved. It was found that a bigger volume of acid is required to dissolve $CaCO_3$ samples containing more magnesium.

The coccolithophore *Emiliania huxleyi* (isolated from the Southern Ocean, strain SO 5.14) was cultured in 0.20 µm filtered natural sea water, with a salinity of 35. Macro- and micro-nutrients were added in excess to ensure supply of all necessary nutrients for growth. The media was continuously bubbled with sterile air supplying carbon dioxide. Cultures received a constant daily illumination of 150 µmol quanta m^{-2} s^{-1} and were harvested at a density of 3,000,000 cells per ml. Samples pellets were produced by centrifugation and subsequently dried and stored at 60 °C till further processing. After rinsing with water, drying in air and weighting the sample (40 mg) was suspended in 9 mL of deionised water. Titration with Cu^{2+} was carried out in suspension to replace the labile Mg (see Electronic Supplementary Information), and then suspended sample was dissolved by addition of nitric acid (0.1 mol L^{-1}) until the pH of the solution was reached 2.0 to ensure complete dissolution of the calcite matrix.

Results and discussion

The minor elements content in calcareous marine microorganisms depends on seawater composition. The literature values of Sr/Ca and Mg/Ca ratios in coccolithic calcite varied from 2.40 to 4.38 mmol mol^{-1} and from 0.066 to 98.6 mmol mol^{-1}, respectively[2]. So, in order to determine the traces of Mg^{2+}, Sr^{2+} in the presence of a huge excess of Ca^{2+}, it is essential to develop highly selective separation of these metals and ideally have both target cations of interest elute prior to the matrix Ca^{2+} peak. Additionally, for application to coccolithophore samples, for the determination of non-labile inorganic magnesium only, the further resolution of an additional divalent cation (in this work Cu^{2+}), added during titration of the sample to release labile organically bound magnesium (see Electronic Supplementary Information), must be achieved. Therefore, herein the separation of Cu^{2+} from Mg^{2+}, Sr^{2+} and excess Ca^{2+} and other possible minor metals from coccolithophore samples was required.

Generally, the affinity to alkali metal cations is very low for most of iminodiacetic acid (IDA) type chelating columns. Hence high alkali metal concentrations originating from the marine microorganism samples should not affect the resultant separation[19]. Additionally, with non-complexing eluents, alkaline earth metals such as Mg^{2+}, Ca^{2+} and Sr^{2+} are generally less retained on such chelating phases than the majority of transition metal ions, including Cu^{2+}[23,25,26]. Therefore herein, particular attention to the eluent conditions were required to pre-elute residual titrated Cu^{2+} ions.

Method development

The separation of metal ions using HEIDA functionalised substrates have been investigated and reported previously[23,27,28,29]. Upon such substrates, both ion-exchange and chelation are the two main interactions simultaneously responsible for the retention selectivity displayed for specific metal ions[19,20]. When using non-complexing acidic eluents, of relatively low ionic strength, simple ion-exchange processes dominate. However, suppression of such interactions through the use of high ionic strength eluents, results in retention from surface chelation becoming the dominant process shaping observed metal ion selectivity[19,20]. For the alkaline earth metals, previous studies[6,24] have shown that with such high ionic strength eluents, the observed selectivity is as follows: $Mg^{2+} < Ba^{2+} < Sr^{2+} < Ca^{2+}$. Generally, nitrates, perchlorates or chlorides of alkali metals are applied to regulate the ionic strength of eluents. Herein, with elevated concentrations of sodium and chloride within the marine derived samples, sodium chloride was obviously the most appropriate eluent additive for controlling ionic strength.

Further control of selectivity can be obtained through the inclusion of complexing ligands within the eluents, to compete with the complexing ligand on the surface of the stationary phase[30]. Recent work[6] using HPCIC for the direct determination of Sr^{2+} in seawater, obtained the separation of Mg^{2+}, Sr^{2+} and Ca^{2+} using an eluent containing glycolic acid (complexing agent) and sodium chloride (ionic strength regulator). However, for the current application, greater resolution of the above metals was required, due to a diverse concentration ratio, and the additional resolution of the alkaline earth metal ions from Cu^{2+}.

In 2008[30], Jones and Nesterenko investigated the effects of different complexing agents as eluent additives. As Cu^{2+} exhibits very strong retention on all IDA based columns, including HEIDA, the use of complexing agents is generally required. Dipicolinic acid and picolinic acid have been proven to provide appropriate affinity to Cu^{2+}, compared to other complexing reagents[30,31], and so were applied in this application. The addition to the eluent of picolinic acid has little effect on Sr^{2+}, Ca^{2+} and Mg^{2+} selectivity, as stability of complexes of these metals with picolinic acid is lower than with the surface bonded HEIDA group in weakly acidic eluents. Alternatively, the β values of Sr^{2+}, Ca^{2+} and Mg^{2+} with dipicolinic acid are higher than that with the surface bonded HEIDA group, and resultant selectivity reflects this.

Using a 0.15 m*M* dipicolinic acid eluent, with 6 m*M* HNO_3 as a starting point, a poor separation of Mg^{2+} and Ca^{2+} achieved, whilst Cu^{2+} failed to elute (**Table 1**). Obviously, a higher concentration of dipicolinic acid was required to elute Cu^{2+}, however, as expected from the high β of Mg^{2+} and Ca^{2+} with dipicolinic acid, increased eluent concentrations of the acid reduced both the retention and resolution of Mg^{2+} and Ca^{2+}, and was therefore unsuitable. A change of complexing agent to picolinic acid, and an increase in concentration (4 m*M*), provided the elution of Cu^{2+}, whilst the separation selectivity (α) between Mg^{2+} and Ca^{2+}, remained relatively unaffected (**Table 1**).

Table 1
The effect of addition of complexing agents to 6 mM HNO_3 used as eluent on retention times (min) and separation selectivity of Mg^{2+}, Ca^{2+} and Cu^{2+}.

Metal ions	Complexing additives		
	0.15 mM dipicolinic acid	2 mM picolinic acid	4 mM picolinic acid
Mg^{2+}	4.69	4.56	4.57
Ca^{2+}	5.13	4.99	5.00
Cu^{2+}	No peak	13.72	6.39
α (Mg^{2+} and Ca^{2+})	1.15	1.16	1.16

Note: "No peak" means strongly retained.

With the addition of sodium chloride (0.25 *M*) and a slight increase of pH (5.0), Mg^{2+}, Ca^{2+} and Cu^{2+} could be separated with retention order of $Mg^{2+} < Ca^{2+} < Cu^{2+}$. However, the elution of Cu^{2+} after Ca^{2+} isn't applicable to analysis of the samples with massive excess of Ca^{2+}. Therefore, a significant increase of picolinic acid concentration (20 m*M*) in the eluent was used to further reduce the retention of Cu^{2+}, whilst a simultaneous reduction in eluent sodium chloride concentration (from 0.25 to 0.1 *M*) provided slightly increased retention of both Mg^{2+} and Ca^{2+}. Under these conditions the desired elution order of $Cu^{2+} < Mg^{2+} < (Cd^{2+}$, potential internal standard cation) $< Sr^{2+} < Ca^{2+}$ was achieved. The optimisation was completed by attenuation of the eluent pH (5.0) and column temperature (40 °C) and resulted in the separation of five metals of interest in less than 10 minutes (see **Figure 1)**.

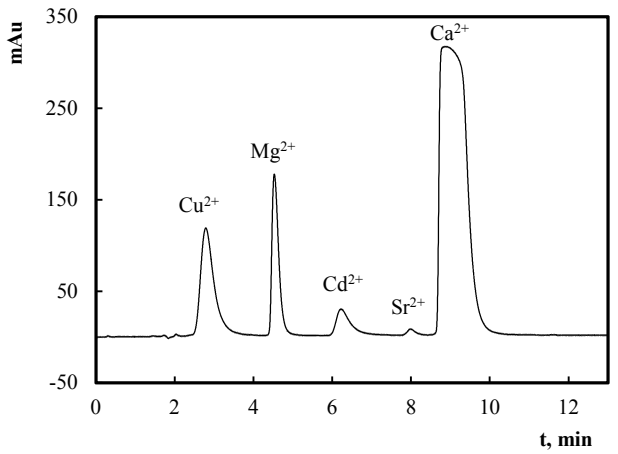


Fig. 1. HPCIC chromatogram of a test mixture of metal cations (Cu^{2+}, Mg^{2+}, Cd^{2+} Sr^{2+}, Ca^{2+}). Column: 100 x 4.6 mm I.D., injection volume 10 µL, flow rate 1 mL min^{-1}, eluent: 20 m*M* picolinic acid, 0.1 *M* NaCl, pH=5. Photometric detection at 490 nm after PCR with ZnEDTA-PAR. PCR reagent flow rate: 0.44 mL min^{-1}.

These conditions provided not only excellent resolution for minor Mg^{2+} and excessive Ca^{2+} peaks, but also a reasonable separation of Sr^{2+} and Ca^{2+} ($\alpha_{Sr2+/Ca2+}$ = 1.15). However, for the latter pair the quantitatve determination of strontium still could be a problem in analysis of real samples (**Figure 2(a)**).
However, according to work[6], the retention of Ca^{2+} on HEIDA functionalised silica is more sensitive to the changes in eluent pH as compared with Mg^{2+} and Sr^{2+}. So, a slight increase in eluent pH to 5.3 improved dramatically the separation selectivity of Sr^{2+} and Ca^{2+} ($\alpha_{Sr2+/Ca2+}$ = 1.30), while the retention of magnesium increased insignificantly (**Figure 2(b)**).

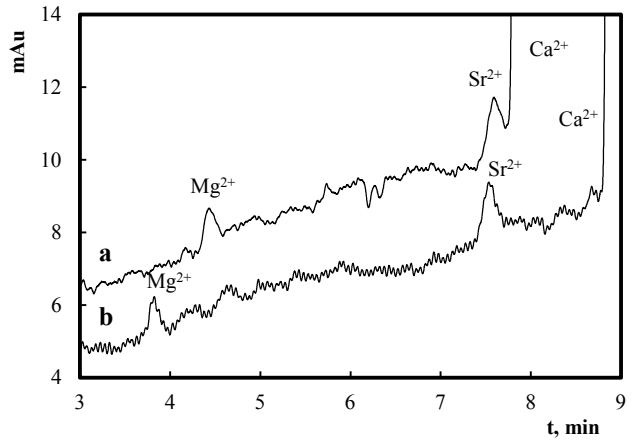


Fig. 2. HPCIC chromatogram of coccolithic calcite model solution (Mg^{2+} 0.024 mg L^{-1}, Sr^{2+} 1.75 mg L^{-1}, Ca^{2+} 400 mg L^{-1}). Column: 100 x 4.6 mm I.D., injection volume 10 µL, flow rate 1 mL min^{-1}, eluent: (a) 20 m*M* picolinic acid, 100 m*M* NaCl, pH=5, (b) 20 m*M* picolinic acid, 80 m*M* NaCl, pH=5.3. Photometric detection at 570 nm after post-column reaction with *o*-CPC. PCR reagent flow rate: 0.44 mL min^{-1}.

Under the above final conditions the retention of a range of divalent and trivalent cations (Ni^{2+}, Mn^{2+}, $Fe^{2+/3+}$, Zn^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+}, Cd^{2+}, Sr^{2+}, Ca^{2+}, and La^{3+}) was also checked to ensure the absence of the possible interference for the determination magnesium and strontium (see **Table 2**).

Detection optimisation

The use of PAR, ZnEDTA-PAR, Calmagite, Arsenazo I, 8-Hydroxyquinolinol or *o*-CPC has been reported as reagents for the photometric detection of alkaline earth metals after PCR in HPCIC[19]. Recently, Nesterenko *et al.*[6] found that ZnEDTA-PAR and *o*-CPC provide the most sensitive photometric detection after PCR for these cations.

Herein, for quantitative analysis, the linear range test for alkaline earth metals was checked for both ZnEDTA-PAR (at pH 10.65) and *o*-CPC (at pH 11.0), with photometric detection at 570 and 490 nm, respectively. The flow rate of the eluent was 1.0 mL min^{-1} and PCR reagent was delivered at a flow rate of 0.44 mL min^{-1}. With *o*-CPC, the linearity was confirmed over the concentration range 1 to 29 mg L^{-1} for Mg^{2+}, 1 to 32 mg L^{-1} for Sr^{2+}, n = 6, with R^2 values = 1.00 and 0.999, respectively. For ZnEDTA-PAR, the linear range was found to be only 0.05 - 4 mg L^{-1} for Mg^{2+} and 0.1 to 32 mg L^{-1} for Sr^{2+}, (n = 6, R^2 = 0.999 for both metals).

The limits of detection (LOD) for Mg^{2+} and Sr^{2+} with each PCR reagent were calculated using the signal to noise criteria S/N = 3. These were found to be 20 µg L^{-1} and 200 µg L^{-1} for *o*-CPC, and 5 µg L^{-1} and 39 µg L^{-1} for ZnEDTA-PAR, for Mg^{2+} and Sr^{2+}, respectively.

In the following experiments Zn-EDTA-PAR was used because of higher sensitivity for Mg^{2+} and $Sr^{2+}$and possibility of detection of various transition metal ions.

Table 2

The retention time (minutes) of metal ions

Metal ions	Ni^{2+}	Mn^{2+}	$Fe^{2+/3+}$	Zn^{2+}	Co^{2+}	Cu^{2+}	Mg^{2+}	Cd^{2+}	Sr^{2+}	Ca^{2+}	La^{3+}
Time (min)	2.18	2.17	1.71/2.18	2.26	2.87	2.93	3.57	5.51	7.12	10.19	No peak

Note: "No peak" means strongly retained

Applications

The accuracy of the proposed method was first verified with the analysis of NIST certified reference material (1d Limestone (Argillaceous)) for the determination of magnesium and strontium. The concentration of Mg^{2+} in the limestone reference material was found to be 1.49 ± 0.04 mg g^{-1}, with *RSD* = 2.4% at *n*=5. This value was 18 % below the certified value 1.82 ± 0.06 mg g^{-1}, so a further confirmatory analysis was completed using sector field HR-ICP-MS. This confirmed a value of 1.49 mg g^{-1} for Mg^{2+}, matching exactly that found using the HPCIC method. The concentration of Sr^{2+} determined within the limestone certified reference material was found to be 0.254 ± 0.005 mg g^{-1} (*RSD* = 2.1%), which matched very well with the certified value 0.256 ± 0.008 mg g^{-1}. The complete data of HR-ICP-MS analysis are presented in Electronic Supplementary Information. The HPCIC chromatogram obtained for the certified reference limestone materials can be seen in **Figure 3**. Traces of Zn^{2+}, Fe^{2+}/Fe^{3+}, and Cu^{2+} were also detected in the limestone sample, but were well resolved from the Mg^{2+} and Sr^{2+} peaks of interest. Technically, copper and zinc can be determined quantitatively in the reference material under these conditions, but it was not under scope of this work.

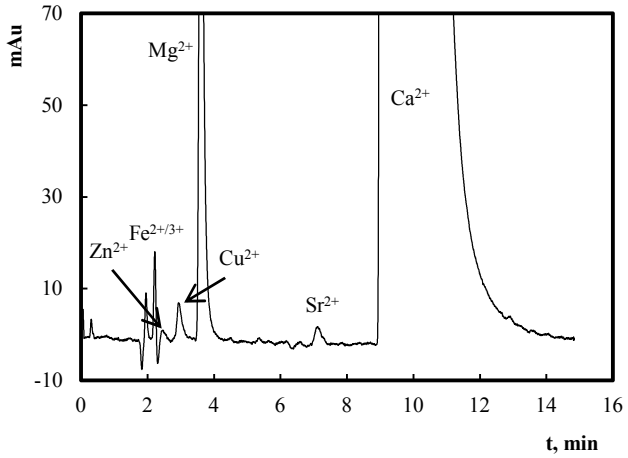


Fig. 3. HPCIC chromatogram of a sample solution of 1 mg mL^{-1} limestone certified reference material dissolved in HNO_3 (0.03*M*). Column: 100 x 4.6 mm I.D., injection volume 10 µL, flow rate 1 mL min^{-1}, eluent: 20 m*M* picolinic acid, 80 m*M* NaCl, pH=5.3. Photometric detection at 490 nm after PCR with ZnEDTA-PAR. PCR reagent flow rate: 0.44 mL min^{-1}. According to the passport of the reference material the sample contains 0.018 µg mL^{-1} Zn^{2+}, 1.82 µg mL^{-1} Mg^{2+}, 0.26 µg mL^{-1} Sr^{2+} and 378 µg mL^{-1} Ca^{2+}.

To evaluate the suitability of the developed method to the determination of Mg^{2+} and Sr^{2+} traces in the samples with high matrix calcium, three laboratory grade $CaCO_3$ samples from different producers were also analysed. **Figure 4(a)** and **4(b)** show the chromatograms resulting from the injection of a 10 mg mL^{-1} solutions prepared from $CaCO_3$ obtained from AJAX and BDH, respectively. In both cases the sample concentrations were high enough to quantitate both Mg^{2+} and Sr^{2+} without loss of resolution between peak of Sr^{2+} and massive peak of Ca^{2+}. However, diluted solution (1 mg mL^{-1}) was prepared for the analysis of $CaCO_3$ sample obtained from Strem Chemicals, Inc. because this contained a significant impurity of Mg^{2+} (see **Figure 4(c)**, which otherwise will be at concentration beyond linearity range. In the latter case, both the peaks for Mg^{2+} and Sr^{2+} impurities can be clearly identified. Using a standard addition method, the concentration of Mg^{2+} in this sample was found to be 5.05 ± 0.04 mg g^{-1}, with *RSD* = 0.76% (*n* = 5). This again matched well with the results of HR-ICP-MS analysis, with which the concentration of Mg^{2+} was found to be 5.10 mg g^{-1}, which corresponds to less than 1% difference between methods.

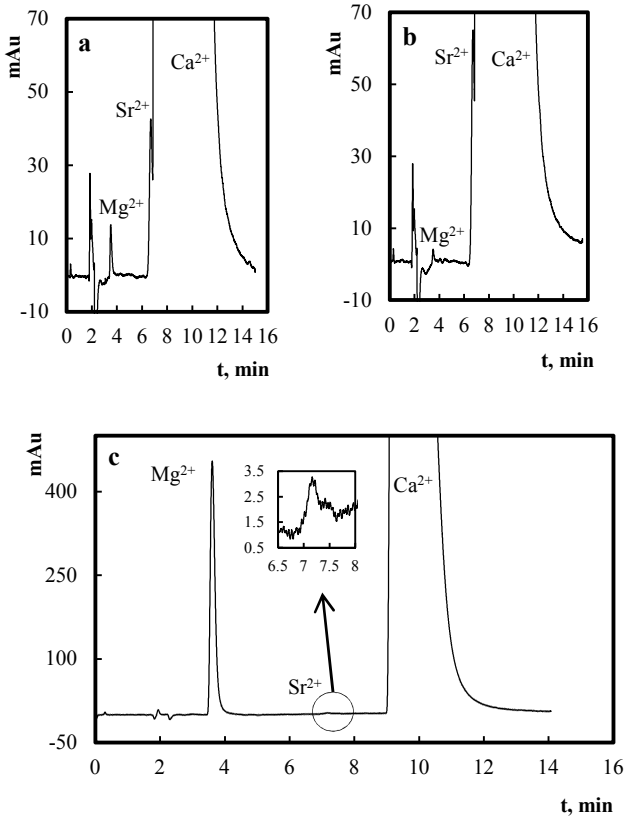


Fig. 4. HPCIC chromatograms of laboratory grade $CaCO_3$ sample solutions. (**a**) 10 mg mL^{-1} $CaCO_3$ (AJAX), dissolved in HNO_3 (0.3*M*). (**b**) 10 mg mL^{-1} $CaCO_3$ (BDH) dissolved in HNO_3 (0.3*M*). (**c**) 1 mg mL^{-1} $CaCO_3$ (Strem Chem.) dissolved in HNO_3 (0.03*M*).

Finally, the developed method was applied to the determination of non-labile inorganic magnesium and the Sr/Ca ratio in coccolithic calcite skeletons (*Emiliania huxleyi*). For this purpose the sample of calcite was pre-titrated using Cu^{2+} to

replace organically bound or physically adsorbed magnesium in coccoliths skeletons. Organic magnesium is mainly presented as chlorophyll and can be substituted in its molecules by copper. The content of inorganic or calcite entrapped magnesium was obtained by subtraction of the concentration of organic magnesium measured in filtrate of the sample titrated by Cu^{2+} from total concentration of magnesium in coccolith. The latter concentration was measured for the sample completely dissolved in nitric acid. The chromatogram of the filtrate of titrated with Cu^{2+} sample is shown in **Figure 5**. The ratio of non-labile inorganic Mg to Ca in coccolithic calcite skeletons was determined to be 5.39 ± 0.23 mmol mol^{-1}, with *RSD* = 4.3 % (*n*=5). This value is in a good agreement with the literature data[2], where Mg/Ca ratio varied in the range 0.066-98.6 mmol mol^{-1}.

Using HPCIC method, the ratio of Sr to Ca in coccolithic calcite skeletons was found to be 2.79 ± 0.22 mmol mol^{-1}, with *RSD* = 7.8 % at *n*=7. This is in a good agreement with 2.73 ± 0.22 mmol mol^{-1} reported in the literature[3] for this sample, when ICP-MS was used for the analysis.

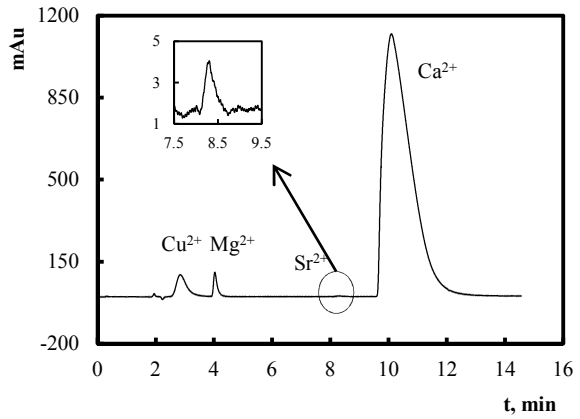


Fig. 5. HPCIC chromatogram of a sample solution of 4.4 mg mL^{-1} coccolithic calcite solution pH = 2, adjusted by HNO_3 (0.1*M*), pre-titrated with Cu^{2+} (60 mg mL^{-1}). The concentrations of Mg^{2+}, Sr^{2+} and Ca^{2+} were 10.28 µg ml^{-1}, 2.48 µg ml^{-1} and 481.45 µg ml^{-1}, respectively. Cu^{2+} was not quantitatively measured as it was the titrant.

Conclusion

A simple and sensitive HPCIC method for the determination of magnesium and strontium in calcite based sample matrices has been developed. The results achieved for the various samples compared well with either certified values or values obtained from HR-ICP-MS. The chromatographic method demonstrated excellent analytical performance, and importantly provides an analytical tool for further study into the important issue of environmental impact upon coccolith shell chemistry.

Acknowledgement

Authors wish to thank Dr. Ashley Townsend from Central Science Laboratory of the University of Tasmania for the ICP-MS analysis. This work was supported by a grant from the Australian Research Council (DP130101518).

References

1 R. L. Folk, *J. Sediment. Res.* 1974, **44**, 40-53.
2 M. N. Müller, M. Lebrato, U. Riebesell, J. Barcelos e Ramos, K. G. Schulz, S. Blanco-Ameijeiras, S. Sett, A. Eisenhauer, H. M. Stoll, *Biogeosciences,* 2014, **11**, 1065–1075.
3 S. Blanco-Ameijeiras, M. Lebrato, H.M. Stoll, M.D. Iglesias-Rodriguez, A. Méndez-Vicente, S. Sett, M. N. Müller, A. Oschlies, K. G. Schulz, *Geochim. Cosmochim. Acta* 2012, **89**, 226-239.
4 M. N. Müller, B. Kısakürek, D. Buhl, R. Gutperlet, A. Kolevica,; U. Riebesell, H. Stoll, A. Eisenhauer, *Geochim. Cosmochim. Acta* 2011, **75**, 2088-2102.
5 T. I. Platzner, I. Segal, L. Halicz, *Anal. Bioanal. Chem.,* 2008, **390**, 441-450.
6 E. P. Nesterenko, P. N. Nesterenko, B. Paull, M. Meléndez, J. Corredor, *Microchem. J.* 2013, **111**, 8-15.
7 V. T. C. Chang, A. Makishima, N. S. Belshaw, R. K. O'Nions, *J. Anal. Atom. Spectrom.,* 2003, 18, 296-301.
8 E. P. Horwitz, M. L. Dietz, D. E. Fisher, *Anal. Chem.,* 1991, **63**, 522-525.
9 E. P. Horwitz, R. Chiarizia, M. L. Dietz, *Solvent Extr. Ion. Exch.,* 1992, **10**, 313-336.
10 J. J. Diamond, *Anal. Chem.,* 1955, **27**, 913-915.
11 R. A. Schmitt, T. A. Linn, H. Wakita, *Radiochim. Acta*, 1970, **13**, 200-212.
12 C. Barber, *Chem. Geol.,* 1974, **14**, 273-280.
13 P. Robinson, *Chem. Geol.,* 1980, **28**, 135-146.
14 B. Ma, Minghui-Chen, Q. Zhang, Qingqing-Yang, Zhendong-Yang, Zimei-Wu, Yanping-Cheng, Yonglu-Wang, Hanjie-Ying, *Afr. J. Biotechnol.,* 2011, **10,** 18039-18045.
15. P. R. Haddad, P. N. Nesterenko, W. Buchberger, *J.Chromatogr.A,* 2008, **1184**, 456-473.
16 C. J. Evenhuis, W. Buchberger, E. F. Hilder, K. J. Flook, C. A. Pohl, P. N. Nesterenko, P. R. Haddad, *J. Sep. Sci.* 2008, **31**, 2598-2604.
17 S. S. Goyal, A. A. R. Hafez, D. W. Rains, *Agron. J.,* 1993, 85, 1192-1197.
18 E. A. Gautier, R. T. Gettar, R. E. Servant, *Anal. Chim. Acta,* 1993, **283**, 350-353.
19 P. N. Nesterenko, P. Jones, B. Paull, *High performance chelation ion chromatography*; RSC Chromatography Monographs, Cambridge, 2011, 303p.
20 P. N. Nesterenko, P. Jones, *J. Sep. Sci.,* 2007, **30**, 1773-1793.
21 P. Jones, M. Foulkes, B. Paull, *J. Chromatogr. A.,* 1994, **673**, 173-179.
22 M. J. Shaw, S. J. Hill, P. Jones, P. N. Nesterenko, *J. Chromatogr. A* 2000, **876**, 127-133.
23 P. N. Nesterenko, P. Jones, *J. Chromatogr. A.,* 1997, **770**, 129-135.
24 M. Meléndez, E. P. Nesterenko, P. N. Nesterenko, J. E. Corredor, *Limnol. Oceanogr.: Methods,* 2013, **11**, 466–474.
25 Á. Moyna, D. Connolly, E. Nesterenko, P. N. Nesterenko, B. Paull, *J. Chromatogr. A.,* 2012, **1249**, 155-163.
26 Á. Moyna, D. Connolly, E. Nesterenko, P. N. Nesterenko, B. Paull, *Anal. Bioanal. Chem.,* 2013, **425**, 2207-2217.

27 E. Sugrue, P. Nesterenko, B. Paull, *J. Sep. Sci.* 2004, **27**, 921-930.
28 N. McGillicuddy, E. P. Nesterenko, P. N. Nesterenko, P. Jones, B. Paull, *J. Chromatogr. A.,* 2013, **1276**, 102-111.
29 N. McGillicuddy, E.P. Nesterenko, P. Jones, D. Caldarola, B. Onida, A.T. Townsend, D. P. Mitev, P. N. Nesterenko, B. Paull, *Anal. Methods*, 2013, **5**, 2666-2673.
30 P. Jones, P. N. Nesterenko, *J. Chromatogr. A.,* 2008, **1213**, 45-49.
31. J. C. Dias, L. T. Kubota, P. N. Nesterenko, G. W. Dicinoski, P. R. Haddad, , *Anal. Methods*, 2010, **2**, 1565-1570.

Notes and references

[a] Australian Centre for Research on Separation Sciences (ACROSS), School of Physical Sciences, University of Tasmania, Australia, email: Pavel.Nesterenko@utas.edu.au.
[b] Address here. Institute for Marine and Antarctic Studies (IMAS), University of Tasmania, Private Bag 129, Hobart, TAS 7001, Australia.
[c] Institute of Oceanography, University of São Paulo, Praça do Oceanográfico 191, 05508-120 São Paulo, SP, Brazil.

† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/