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

Yan Li,a Brett Paull,a Marius N. Müller,b 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 × 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 mM NaCl and 20 mM picolinic acid at pH 5.30 was found to provide complete separation of Ni²⁺, Cu²⁺, Mg²⁺, Cd²⁺, Sr²⁺ and Ca²⁺ in 15 minutes, in matrices with 20 000 fold excess of Ca²⁺. 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 Mg²⁺ (LOD 20 µg L⁻¹ and 5 µg L⁻¹ for o-CPC and ZnEDTA-PAR, respectively) and Sr²⁺ (LOD 200 µg L⁻¹ and 39 µg L⁻¹ for o-CPC and ZnEDTA-PAR, respectively). Using o-CPC, the linear range was from 0.5 to 24 mg L⁻¹ for Mg²⁺ and from 1.0 to 32 mg L⁻¹ for Sr²⁺. For ZnEDTA-PAR, the linear range was from 0.5 to 4 mg L⁻¹ for Mg²⁺ and 0.1 to 32 mg L⁻¹ for Sr²⁺. 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. The 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 in a variety of scientific fields, particularly for 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 utilized to provide insight into the nature of ancient oceans and environments. Furthermore, the rate of inorganic minor element incorporation (e.g. Mg and Sr) into calcium enriched matrices such as coccolithophore calcite can be of great significance for the evaluation of changes in the current marine environment,4 including changes associated with global warming and increasing CO₂ emissions. However, in the latter case, complicating factors associated with removal of residual sea salts and organically bound magnesium from coccolithophore calcite samples5 means that currently there are few practical analytical methods for the accurate determination of trace inorganic magnesium, present as MgCO₃, in such materials. In addition, the excess of C and Ca within all forms of calcite can cause significant isobaric interference for the precise determination of trace Mg²⁺ and Sr²⁺ (e.g. limestone6) when using techniques such as inductively coupled plasma mass spectrometry (ICP-MS).6 The measurement of magnesium isotopes (²⁵Mg⁺, ²⁶Mg⁺ and ²⁷Mg⁺) can suffer from isobaric interference from carbon-dimer ions (¹²C₂⁺, ¹²C₁³C⁺ and ¹³C₂⁺). Similarly, strontium isotopes, ⁸⁸Sr, ⁸⁶Sr, ⁸⁷Sr and ⁸⁸Sr, can suffer from isobaric interference from Ca-dimer ion signals (⁴⁰Ca₁⁴Ca⁺, ⁴²Ca₂⁺, ⁴⁰Ca₁⁶Ca⁺, ⁴²Ca₁⁸Ca⁺, ⁴³Ca₂⁺, ⁴⁰Ca₁⁸Ca⁺, ⁴²Ca₁⁶Ca⁺, and ⁴⁴Ca₂⁺). To fully eliminate such interference, it is often necessary to apply additional chromatographic separation of these elements prior to introduction into the ICP-MS. For example, Chang et al. determined magnesium in CaCO₃ (ref. 7) using multiple collector ICP-MS, by including a two-step ion-exchange chromatographic pre-treatment procedure. Likewise, extraction chromatography with polycyacrylate porous resin coated by a 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.59 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,18 neutron activation analysis (NAA)19 and atomic absorption spectroscopy (AAS).20,21 However, these methods also have their limitations. For example, the linear range of both atomic emission and absorption spectroscopy is rather narrow,22,23 and due to the high ionic strength and complexity of samples, analysis generally requires the removal of the 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,24 and has been widely used for the determination of Mg and Sr in various complex sample matrices, including seawater,16 plant-tissues17 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 are 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 to 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 et al.21 published the determination of Ba2+ and Sr2+ in calcium-containing matrices using HPCIC. However, this chromatographic system could not separate Mg2+ from the huge amount of Ca2+. In addition, since the technique was in its infancy at that time and column limitations, the whole separation took an excessive 50 minutes. Recent studies reported much faster (5-15 min) determination of Be2+ in stream sediments,22 and Mg2+, Ca2+ and Sr2+ in seawater23,24 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 on hydroxyethyliminodiacetic acid (HEIDA) bonded monolithic silica columns and optimised post-column reaction (PCR) detection. The 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 inorganic 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, a peristaltic pump, a UV-Vis detector and a post-column reactor (Metrohm, Herisau, Switzerland). The post-column reactor consisted of a plastic tee and a 2.5 m long PTFE capillary reaction coil. Two polyetheretherketone (PEEK) sample loops, 10 µL and 20 µL, were used, with manual sample injection. ICNet 2.3 SR6 software (Metrohm, Herisau, Switzerland) was used for IC data acquisition and processing of chromatograms. A 100 x 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 reagents**

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)resorcinal (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 (Na2B4O7·10H2O, 99.5%) and ZnEDTA were purchased from BDH chemicals (Poole, UK). CaCO3 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 Ni2+, Fe2+/3+, Ca2+, Sr2+, Mg2+, Mn2+, Co2+, Cd2+, Zn2+, La3+ and Cu2+, 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 the National Institute of Standards and Technology, NIST, USA. Full details of its composition can be found at the 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 mM o-CPC, 0.25 M boric acid adjusted to pH 11 using NaOH; (2) 0.15 mM PAR, 0.4 M NH4OH, adjusted to pH 10.65 with nitric acid; (3) 0.2 mM ZnEDTA and 0.15 mM PAR in 2 M NH4OH, adjusted to pH 10.65.
Sample preparation

For CaCO₃ and limestone samples, nitric acid (30 mM, 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 that all the carbonates were fully dissolved. It was found that a bigger volume of acid is required to dissolve CaCO₃ samples containing more magnesium.

The coccolithophore *Emiliania huxleyi* (isolated from the Southern Ocean, strain EHSO 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 the supply of all necessary nutrients for growth. The medium 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. Sample pellets were produced by centrifugation and subsequently dried and stored at 60 °C till further processing.

After rinsing with water, drying in air and weighing the sample (40 mg) was suspended in 9 mL of deionised water. Titration with Cu²⁺ was carried out in suspension to replace the labile Mg (see the ESI†), and then the suspended sample was dissolved by addition of nitric acid (0.1 mol L⁻¹) until the pH of the solution reached 2.0 to ensure complete dissolution of the calcite matrix.

Results and discussion

The minor element 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 Mg²⁺ and Sr²⁺ in the presence of a huge excess of Ca²⁺, it is essential to develop highly selective separation of these metals and ideally have both target cations of interest elute prior to the matrix Ca²⁺ 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 Cu²⁺), added during titration of the sample to release labile organically bound magnesium (see the ESI†), must be achieved. Therefore, herein the separation of Cu²⁺ from Mg²⁺, Sr²⁺ and excess Ca²⁺ and other possible minor metals from coccolithophore samples was required.

Generally, the affinity to alkali metal cations is very low for most of the iminodiacetic acid (IDA) type chelating columns. Hence high alkali metal concentrations originating from the marine microorganism samples should not affect the resultant separation.³ Additionaly, with non-complexing eluents, alkaline earth metals such as Mg²⁺, Ca²⁺ and Sr²⁺ are generally less retained on such chelating phases than the majority of transition metal ions, including Cu²⁺.³,²³,²⁷,²⁸ Therefore herein, particular attention to the eluent conditions was required to pre-elute residual titrated Cu²⁺ ions.

Method development

The separation of metal ions using HEIDA functionalised substrates has been investigated and reported previously.²³,²⁷–²⁹ Upon such substrates, both ion-exchange and chelation are the two main interactions simultaneously responsible for the retention selectivity displayed for specific metal ions.¹⁹,²⁹ When using non-complexing acidic eluents, of relatively low ionic strength, simple ion-exchange processes dominate. The suppression of ion-exchange interactions by using eluent with high ionic strength results in dominance of surface chelation in the retention mechanism and in a higher separation selectivity.¹⁹,²⁹ For the alkaline earth metals, previous studies⁶,²⁴ have shown that with such high ionic strength eluents, the observed selectivity is as follows: Mg²⁺ < Ba²⁺ < Sr²⁺ < Ca²⁺. 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.⁸ Recent work⁶ using HPCIC for the direct determination of Sr²⁺ in seawater demonstrated the separation of Mg²⁺, Sr²⁺ and Ca²⁺ 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 Cu²⁺.

In 2008,⁸ Jones and Nesterenko investigated the effects of different complexing agents as eluent additives. As Cu²⁺ 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 Cu²⁺, compared to other complexing reagents,²⁸,³¹ and so were applied in this application. The addition of picolinic acid to the eluent has little effect on the separation selectivity of Sr²⁺, Ca²⁺ and Mg²⁺, as the stability of complexes of these metals with picolinic acid is lower than that of complexes formed with the surface bonded HEIDA group. Alternatively, the β values of Sr²⁺, Ca²⁺ and Mg²⁺ with dipicolinic acid are higher than that with the surface bonded HEIDA group, and resultant selectivity reflects this.

Using a 0.15 mM dipicolinic acid eluent, with 6 mM HNO₃, as a starting point, a poor separation of Mg²⁺ and Ca²⁺ was achieved, whilst Cu²⁺ failed to elute (Table 1). Obviously, a higher concentration of dipicolinic acid was required to elute Cu²⁺, however, as expected from the high β of Mg²⁺ and Ca²⁺ with dipicolinic acid, increased eluent concentrations of the acid reduced both the retention and resolution of Mg²⁺ and Ca²⁺, and was therefore unsuitable. A change of complexing agent to picolinic acid and an increase in concentration (4 mM) provided the elution of Cu²⁺, whilst the separation selectivity (α) between Mg²⁺ and Ca²⁺ remained relatively unaffected (Table 1).

With the addition of sodium chloride (0.25 M) and a slight increase of pH (5.0), Mg²⁺, Ca²⁺ and Cu²⁺ could be separated with a retention order of Mg²⁺ < Ca²⁺ < Cu²⁺. However, the elution of Cu²⁺ after Ca²⁺ is not applicable to the analysis of the samples with massive excess of Ca²⁺. Therefore, a significant
increase of picolinic acid concentration (20 mM) in the eluent was used to further reduce the retention of Cu²⁺, whilst a simultaneous reduction in eluent sodium chloride concentration (from 0.25 to 0.1 M) provided slightly increased retention of both Mg²⁺ and Ca²⁺. Under these conditions the desired elution order of Cu²⁺ < Mg²⁺ < (Cd²⁺, potential internal standard cation) < Sr²⁺ < Ca²⁺ 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 Fig. 1).

These conditions provided not only excellent resolution for minor Mg²⁺ and excessive Ca²⁺ peaks, but also a reasonable separation of Sr²⁺ and Ca²⁺ (α_{Sr²⁺/Ca²⁺} = 1.15). However, for the latter pair the quantitative determination of strontium still could be a problem in the analysis of real samples (Fig. 2(a)). However, according to work, the retention of Ca²⁺ on HEIDA functionalised silica is more sensitive to the changes in eluent pH as compared with Mg²⁺ and Sr²⁺. So, a slight increase in eluent pH to 5.3 improved dramatically the separation selectivity of Sr²⁺ and Ca²⁺ (α_{Sr²⁺/Ca²⁺} = 1.30), while the retention of magnesium increased insignificantly (Fig. 2(b)).

Under the above final conditions the retention of a range of divalent and trivalent cations (Ni²⁺, Mn²⁺, Fe²⁺/³⁺, Zn²⁺, Co²⁺, Cu²⁺, Mg²⁺, Cd²⁺, Sr²⁺, Ca²⁺, and La³⁺) was also checked to ensure the absence of the possible interference for the determination of magnesium and strontium (see Table 2).

### Table 1 The effect of addition of complexing agents to 6 mM HNO₃ used as an eluent on the retention times (min) and separation selectivity of Mg²⁺, Ca²⁺ and Cu²⁺

<table>
<thead>
<tr>
<th>Metal ions</th>
<th>Complexing additives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.15 mM dipicolinic acid</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>4.69</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>5.13</td>
</tr>
<tr>
<td>Cu²⁺</td>
<td>No peak</td>
</tr>
<tr>
<td>α (Mg²⁺ and Ca²⁺)</td>
<td>1.15</td>
</tr>
</tbody>
</table>

α Note: “No peak” means strongly retained.

### Detection optimisation

The use of PAR, ZnEDTA-PAR, calmagite, arsenazo I, 8-hydroxquinolinol or o-CPC has been reported as reagents for the photometric detection of alkaline earth metals after PCR in HPCIC. Recently, Nesterenko et al. 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 of 1 to 29 mg L⁻¹ for Mg²⁺, 1 to 32 mg L⁻¹ for Sr²⁺, 1 to 4 mg L⁻¹ for Ca²⁺, and 1 to 0.3 mg L⁻¹ for Cd²⁺.
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 (LODs) 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 mL$^{-1}$ and 200 μg mL$^{-1}$ 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 ZnEDTA-PAR was used because of higher sensitivity for Mg$^{2+}$ and Sr$^{2+}$ and the possibility of detection of various transition metal ions.

**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 the ESI.† The HPCIC chromatogram obtained for the certified reference limestone materials can be seen in Fig. 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.

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. Fig. 4(a) and (b) show the chromatograms resulting from the injection of a 10

### Table 2  The retention time (minutes) of metal ions$^a$

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

$^a$ Note: “No peak” means strongly retained.
mg mL\(^{-1}\) solution 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 the peak of Sr\(^{2+}\) and the massive peak of Ca\(^{2+}\). However, a diluted solution (1 mg mL\(^{-1}\)) was prepared for the analysis of the CaCO\(_3\) sample obtained from Strem Chemicals, Inc. because this contained a significant impurity of Mg\(^{2+}\) (see Fig. 4(c)), which otherwise will be at concentration beyond the 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.

Finally, the developed method was applied to the determination of non-labile inorganic magnesium and the Sr/Ca ratio in coccolith 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 coccolithic 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 the filtrate of the sample titrated with 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 the sample titrated with Cu\(^{2+}\) is shown in Fig. 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 good agreement with the literature data,\(^4\) where the Mg/Ca ratio varied in the range of 0.066–98.6 mmol mol\(^{-1}\).

Using the 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% \((n = 7)\). This is in good agreement with 2.73 ± 0.22 mmol mol\(^{-1}\) reported in the literature\(^6\) for this sample, when ICP-MS was used for the analysis.

### 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 demonstrates excellent analytical performance, and importantly provides an analytical tool for further study into the important issue of environmental impact upon coccolith shell chemistry.

### Acknowledgements

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