Chemical Geology 437 (2016) 1–6

Contents lists available at ScienceDirect

Chemical Geology

journal homepage: www.elsevier.com/locate/chemgeo

Precise and fast determination of inorganic magnesium in coccolithophore calcite

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ARTICLE INFO

Article history:
Received 7 October 2015
Received in revised form 29 April 2016
Accepted 9 May 2016
Available online 11 May 2016

Keywords:
Phytoplankton
Coccolithophore
Calcite
Magnesium
Strontium
Determination

ABSTRACT

Coccolithophores are calcifying marine phytoplankton playing a pivotal role in the production of calcium carbonate deposits. During the formation of calcium carbonate phase, calcium ions may be replaced by other divalent cations (e.g. Sr^{2+} and Mg^{2+}) which results in a unique elemental signature showing environmental and, in case of biogenic precipitates, physiological conditions of its formation. It was shown that the ratio between magnesium and calcium is strongly correlated with surface sea temperature variation and can be used in paleoceanographic studies to reconstruct past environmental conditions and to understand biogeochemical cycles. The determination of the coccolithophore calcite Mg/Ca ratio, however, requires the efficient removal or quantification of organically bound magnesium, which is up to 400 times more abundant than the magnesium incorporated within the inorganic calcite. Classical methods, using oxidation to remove organic matter, are time consuming and require a considerable amount of sample material. So, the aim of our investigation is the development of a new reliable method for determination of inorganic magnesium and the Mg/Ca ratio in coccolithophore calcite. For this purpose, labile organic bound magnesium is replaced by the incubation with added Cu^{2+} for 10 min, and the released Mg^{2+} is determined via high performance chelation ion chromatography (HPCIC). This method enabled the determination of the coccolithophore calcite Mg/Ca ratio within 1 h using <5 mg dry coccolithophore sample material. The method has been successfully tested with laboratory cultured calcifying and non-calcifying coccolithophores samples. The portable HPCIC instrumentation can be easily mounted on-board scientific research vessels and thus potentially represent a new tool for in-situ calcite analysis of coccolithophore surface bloom situations. However, a further work is required to make this method suitable for analysis of pelagic sediments and sediment traps, which may contain residues of Mg-rich clays.

1. Introduction

Coccolithophores are unicellular marine calcifying phytoplankton, which are a key contributor to the pelagic production and deposition of calcium carbonate. The sediment record of coccolithophores dates back to the Triassic and has been subject to paleoceanographic studies (Bolton et al., 2012; Hermoso, 2014; Hermoso, 2016; Rousselle et al., 2013; Stoll and Ziveri, 2004). The biogenic precipitated calcium carbonate contains other divalent cations replacing the calcium ion in the crystal structure. The ratio between the trace elements and calcium (e.g. Sr/Ca and Mg/Ca) are widely applied as paleoproxies to reconstruct past oceanographic conditions and a careful calibration is necessary to account for possible biological or vital effects (Müller et al., 2014; Stoll et al., 2002a). Before we can generate meaningful coccolith Mg/Ca ratios, it is essential to eliminate Mg from non-carbonated sources in order to determinate precisely the amount of Mg entrapped into the calcite crystal. Organically bound Mg, which is up to 400 times more abundant in coccolithophore cells than inorganically bound Mg (Rosenthal et al., 2000), is found in cellular polyphosphate compounds and biomolecules (e.g. chlorophyll) and acts as a cofactor with ATP to activate numerous enzymatic reactions (Li et al., 2001).

All present methods for the determination of magnesium in coccolithophore calcite consider removal of organically bound magnesium (Holcomb et al., 2015). These methods include oxidation of organic phase with either sodium hypochlorite (Bairbakhish et al., 1999; Blanco-Ameijeiras et al., 2012; Stoll et al., 2001) or with hydrogen peroxide (Blanco-Ameijeiras et al., 2012). In the latter case the oxidation of the sample by alkaline hydrogen peroxide (H_2O_2 + NaOH) is applied according to a widely-used cleaning method for foraminifera (Barker et al., 2005; Barker et al., 2003; Boyle, 1983; Martin and Lea, 2002). These methods have changed little since they were developed and the majority of these methods are technically complex as well as labour- and time-consuming (Blanco-Ameijeiras et al., 2012; Stoll et al., 2001). Additionally, the reaction of inorganic magnesium and calcium with organic acids, formed as intermediate oxidation products of organic

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http://dx.doi.org/10.1016/j.chemgeo.2016.05.010
0009-2541/

substances, potentially contributes to the systematic error of classical oxidation methods (Holst, 1954). The Mg/Ca and Sr/Ca ratios can be evaluated for the purified inorganic materials by using ICP-MS or ICP-AES, which require the efficient removal of sea salts and any other contaminating phases to reduce matrix interference. The presence of the excess of Ca^{2+} can cause significant isobaric interferences for the determination of specific elements at trace level by ICP-MS (Nesterenko et al., 2012). Especially the precise determination of trace Sr and its isotopes (^{84}Sr, ^{86}Sr, ^{87}Sr and ^{88}Sr) can suffer isobaric interference from Ca-dimer ions signals and argide ions (Nesterenko et al., 2012; Platzner et al., 2008).

In this study, a new method for the separation and determination of inorganic and organic magnesium in coccolithophore sample material was developed. The method includes the replacement of organically labile Mg^{2+} by addition of Cu^{2+}, followed by the quantitative determination of organic and inorganic Mg using high performance chelation ion chromatography (HPCIC). The mild conditions of our method allow removal of only labile or reactive Mg^{2+} from organic phase of coccolith and minimise the possible errors due to reaction of inorganic matrix with organic acids formed during oxidation of organic phases. The corresponding HPCIC methods were developed previously for the determination of trace Mg and Sr in calcite (Li et al., 2015) and in sea water (Meléndez et al., 2013; Nesterenko et al., 2012). In addition, new insights into the binding mechanism of the organically bound Mg^{2+} in coccolithophores are discussed. The optimised new procedures were applied to coccolithophore samples produced from laboratory culture experiments. The developed methodology and portable analytical instrumentation could be easily used in on-board experimental work for the determination of coccolithophore calcite Mg/Ca ratios in coccolithophore surface bloom situations. However, an application of this method for analysis marine sediments may require a further development to eliminate the contribution of Mg-rich clay minerals, which cannot be trivially separated from coccolith ooze (Halloran et al., 2009; Minoletti et al., 2009; Stoll et al., 2007a).

2. Materials and methods

2.1. Reagents and solutions

Analytical or higher purity grade reagents and deionised water (Millipore, Bedford, MA, USA) were used for the preparation of all solutions. *o*-Cresolphthalein complexone (*o*-CPC) or 3,3′-bis[*N*,*N*-di-(carboxymethyl)aminomethyl]-*o*-cresolphthalein (90% dye content) was supplied by 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%), potassium chloride (KCl, 99.5%) 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 was purchased from BDH chemicals (Poole, UK). Calcium carbonate calcite ($CaCO_3$, 99%) was purchased from Strem Chemicals (Miami, USA).

Nitric acid (69%) and ammonium hydroxide (25%) were obtained from Merck (Sydney, Australia). Boric acid and Spectrosol atomic absorption standard solutions of Ca^{2+}, Sr^{2+}, Mg^{2+}, Cd^{2+} and Cu^{2+}, with concentrations of 1000 mg L^{-1} were purchased from BDH Chemicals (Poole, UK).

2.2. Instrumentation

For the chromatographic studies and sample analysis, a Model 844 Compact IC with built in high pressure mobile phase pump, reagent peristaltic pump, post-column reactors and spectrophotometric detector with dual wavelength detection at 490 nm and 570 nm was used. Samples were injected with a 10 μL polyetheretherketone (PEEK) manual sample loop. A 100 × 4.6 mm I.D. silica monolithic column with bonded *N*-hydroxyethyliminodiacetic acid (HEIDA) groups was purchased from Phenomenex (Cheshire, UK) and modified according to the procedure described by Sugrue et al. (2004). ICNet 2.3 SR6 software (Metrohm, Herisau, Switzerland) was used for data acquisition and processing of chromatograms. A Soniclean benchtop ultrasonic cleaner (250T, Adelaide, AU) and a Griffin & George LTD (UK) centrifuge were used for sample preparation. Dissolved inorganic carbon (C_T) and total alkalinity (A_T) were analysed based upon the mean of triplicate measurements, applying the infrared detection method with an Apollo SciTech DIC-Analyzer (Model AS-C3), and the potentiometric titration method (Dickson et al., 2003), respectively. Analytical data was validated using reference to Certified Reference Materials (CRM; Scripps Institution of Oceanography). Solubility coefficients for Cu^{2+}, Mg^{2+}, Cd^{2+} and CO_3^{2-} in the sample solution were provided using Medusa 3.2 Chemical Equilibrium Diagrams (see Supplementary information (ESI)).

2.3. Preparation of post-column reagents for HPCIC

Stock solutions of PAR and *o*-CPC, reagents containing 1 mmol L^{-1} PAR in 5 mmol L^{-1} ammonium hydroxide and 2 mmol L^{-1} of *o*-CPC in 0.25 mol L^{-1} boric acid, were used to prepare the post-column reagents. The pH of the stock solutions was high (pH > 10) to prevent adsorption of organic reagents on the plastic surface. Under these conditions the stock solutions are stable for several months without any colour change indicating possible degradation or contamination of the reagent by metals.

Two standard post-column reagents for the detection of alkaline earth metal cations and transition metal cations were used: (1) 0.4 mmol L^{-1} *o*-CPC with 0.25 mol L^{-1} boric acid adjusted to pH 11 using NaOH, (2) 0.2 mmol L^{-1} Zn-EDTA with 0.12 mmol L^{-1} PAR in 2 mol L^{-1} NH_4OH adjusted to pH 10.65 with HNO_3.

2.4. Coccolithophore culture conditions and sample collection

Bulk coccolithophore sample material, which was used to develop the method, was produced in the laboratory from calcifying (strain EHSO 5.14) and non-calcifying strains of *Emiliania huxleyi* (strain EHSO 6.13, provided by the culture collection of the Institute for Marine and Antarctic Studies at the University of Tasmania). Cultures were grown in 0.20 μm filtered natural seawater with a salinity of 35 at 14 °C in 5 L sterile Erlenmeyer flasks. Macro- and micro-nutrients were added in excess to ensure supply of all necessary nutrients for growth (f/2 medium after (Guillard, 1975)). Cultures were constantly bubbled with sterile air, received a constant daily illumination of 150 μmol photons m^{-2} s^{-1} and were harvested at approximately 1×10^6 cells mL^{-1}. Culture cell density was determined by triplicate measurements using a Beckman Coulter Multisizer™ 4 and cellular growth rate, μ (d^{-1}), was calculated as: $\mu = (\ln c_1 - \ln c_0) / (t_1 - t_0)$ where c_0 and c_1 are the cell concentrations at the beginning (t_0) and end of the incubation period (t_1), expressed in days.

Additional sample material was prepared from a calcifying strain of *E. huxleyi* (EHSO 5.14) that was cultured at 4 different pCO_2 levels (270, 470, 800 and 1700 μatm with a relative standard deviation of 8%, Table 1) without air bubbling in a closed system at experimental conditions as described above. Target pCO_2 levels were obtained by the addition of calculated amounts of $NaHCO_3$, HCl and NaOH what resulted in the corresponding $pH_{(total\ scale)}$ values of 8.20, 8.00, 7.80 and 7.49, respectively. This culture experiment followed standard dilute batch culture protocols (Müller et al., 2015; Riebesell et al., 2010). These cultures were harvested at cell densities between 2.5 and 5×10^4 cells mL^{-1} to avoid major changes in the seawater carbonate chemistry due to biological activity. Carbonate chemistry was calculated from initial C_T, A_T, temperature and salinity using CO2sys (v. 1.05 by E. Lewis and D. W. R. Wallace), with the stoichiometric equilibrium constants for carbonic acid given in Roy et al. (1993).